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# Canadian Journal of Botany

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## PRODUCTION OF VITAMIN $B_{12}$ BY *RHIZOBIUM* SPECIES[1]


BY MARGARET O. BURTON[2] AND A. G. LOCHHEAD[3]



### Abstract

A study was made of the capacity for synthesis of vitamin $B_{12}$ by 70 strains representing six species of *Rhizobium*, namely, *R. meliloti*, *R. trifolii*, *R. leguminosarum*, *R. japonicum*, *R. lupini*, and *R. phaseoli*. *Rhizobium meliloti* was sharply distinguished from the other species by its ability to produce significantly higher quantities of the vitamin, one strain producing, under the experimental conditions, over 1000 mμgm. per ml. of culture liquid. The remaining species did not show significant differences in $B_{12}$ synthesizing capacity. No correlation was noted between vitamin-producing capacity and effectiveness of the individual strains with respect to nitrogen fixation.


Recent studies on the production of vitamin $B_{12}$-active substances by microorganisms indicated that for the isolation of active forms a simple medium without growth factors was superior to more complex media, although the latter were more suitable for vitamin production (1). This observation led to a study of vitamin $B_{12}$ production by known cultures of soil bacteria having relatively simple nutrient requirements and the present report deals with a survey of nitrogen-fixing organisms representative of the genera *Rhizobium* and *Azotobacter*.

### Experimental

The organisms were cultured for $B_{12}$ production in 200-ml. Erlenmeyer flasks containing 50 ml. of medium, inoculated with 0.3 ml. of a cell suspension from a 48-hr. agar slant of the corresponding medium in 2.0 ml. saline. The cultures were incubated at 26° C. on a shaking machine for six days, after which the culture broths were adjusted to pH 4.0, heated for five minutes at 15 lb. pressure, and assayed for vitamin $B_{12}$ by the proposed U.S.P. microbiological procedure with *Lactobacillus leichmannii* ATCC 7830 as test organism.

Preliminary studies were made with eight cultures of *Azotobacter*, representing three species, and with 35 cultures of *Rhizobium* which included strains capable of infecting 14 different legume crops. Vitamin production by *Azotobacter* was in all cases low, with average yields of 5 mμgm. per ml. on Starkey's medium and 17 mμgm. per ml. on a casamino acids medium


[1] *Manuscript received April 23, 1952.*

*Contribution No. 338 from the Division of Bacteriology and Dairy Research, Science Service, Department of Agriculture, Ottawa, Canada.*



[2] *Assistant Bacteriologist.*

[3] *Chief, Division of Bacteriology and Dairy Research.*



[The July number of this Journal (Can. J. Botany, 30 : 349-520. 1952.) was issued July 16, 1952.]

TABLE I

VITAMIN $B_{12}$ PRODUCTION BY 70 STRAINS OF *Rhizobium* IN CASAMINO ACIDS MEDIUM *A*

| Cult No. | pH | Optical density* | $B_{12}$ mμgm./ml. | Cult. No. | pH | Optical density | $B_{12}$ | Cult. No. | pH | Optical density | $B_{12}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Rhizobium meliloti* | | | | *Rhizobium japonicum* | | | | *Rhizobium trifolii* | | | |
| 309 | 7.5 | .320 | 477 | 307 | 7.4 | .400 | 210. | 174 | 8.3 | .025 | 8.2 |
| 340 | 7.2 | .210 | 438 | 308 | 7.25 | .210 | 130. | 310 | 5.6 | .046 | 32. |
| 524 | 6.5 | .186 | 285 | 404 | 7.5 | .136 | 90. | 311 | 5.6 | .105 | 140. |
| 756 | 7.2 | .135 | 420 | 460 | 7.25 | .160 | 46. | 525 | 7.0 | .290 | 62. |
| 757 | 5.5 | .208 | 780 | 461 | 8.0 | .025 | 19. | 761 | 7.85 | .015 | 82. |
| 758 | 4.9 | .235 | 450 | 462 | 8.45 | .056 | 57. | 762 | 7.90 | .018 | 4.4 |
| 759 | 5.1 | .202 | 394 | 771 | 7.45 | .041 | 100. | 763 | 7.35 | .020 | 9.8 |
| 760 | 7.35 | .200 | 680 | 772 | 8.3 | .096 | 48. | 764 | 7.0 | .018 | 1.6 |
| 785 | 7.25 | .160 | 301 | 773 | 7.9 | .043 | 34. | 765 | 7.2 | .016 | 3.0 |
| 786 | 4.35 | .130 | 120 | 774 | 5.8 | .081 | 70. | 789 | 6.9 | .015 | 3.2 |
| 787 | 6.65 | .229 | 290 | 798 | 7.8 | .066 | 44. | 790 | 6.6 | .008 | 1.7 |
| 788 | 6.7 | .230 | 290 | 799 | 8.1 | .040 | 46. | 791 | 6.95 | .022 | 4.4 |
| | | | | 800 | 7.7 | .011 | 7.8 | 792 | 7.2 | .088 | 19. |
| *Rhizobium leguminosarum* | | | | *Rhizobium phaseoli* | | | | *Rhizobium lupini* | | | |
| 175 | 8.3 | .026 | 17. | 219 | 5.5 | .035 | 42. | 221 | 7.3 | .023 | 9.6 |
| 313 | 8.5 | .090 | 8.8 | 314 | 8.0 | .012 | 9.9 | 583 | 6.8 | .011 | 0.8 |
| 780 | 6.8 | .015 | 7.6 | 315 | 5.2 | .084 | 64. | 584 | 7.4 | .013 | 2.6 |
| 781 | 8.3 | .024 | 61. | 775 | 5.9 | .027 | 32. | 586 | 6.6 | .018 | 0.4 |
| 782 | 7.0 | .170 | 75. | 776 | 6.7 | .016 | 1.5 | 766 | 5.6 | .125 | 9.5 |
| 783 | 7.0 | .013 | 1.9 | 777 | 7.5 | .030 | 40. | 767 | 6.8 | .011 | 0.3 |
| 784 | 8.1 | .164 | 48. | 778 | 7.9 | .017 | 4.9 | 768 | 7.5 | .016 | 0.7 |
| 793 | 7.5 | .022 | 12. | 779 | 6.7 | .020 | 0.7 | 769 | 4.5 | .045 | 1.4 |
| 794 | 7.7 | .018 | 47. | 795 | 7.15 | .015 | 2.8 | 770 | 5.1 | .024 | 2.4 |
| | | | | 796 | 8.55 | .054 | 34. | 801 | 7.2 | .017 | 2.3 |
| | | | | 797 | 5.1 | .079 | 12. | 802 | 7.2 | .016 | 2.3 |
| | | | | | | | | 803 | 7.3 | .040 | 9.8 |

* *Culture diluted 1-30.*

(Medium *A* as described below) with the addition, in all cases of cobaltous chloride. On the other hand, the *Rhizobium* strains varied greatly in $B_{12}$ production, showing yields ranging from 0.7 to 300 m$\mu$gm. per ml. in Medium *A*, which was generally superior to a yeast–mannitol medium for production of the vitamin. Some correlation between the yield and species was noted, the highest production being shown by *Rhizobium meliloti*.

Since the results obtained with the rhizobia warranted further study, tests were made with 70 strains representing the following six species: *R. meliloti*, *R. trifolii*, *R. leguminosarum*, *R. japonicum*, *R. lupini*, and *R. phaseoli*. The cultures chosen varied widely with respect to their nitrogen-fixing ability. Vitamin $B_{12}$ production was determined in two cultural media:

*Medium A:* potassium monohydrogen phosphate, 0.5 gm.; potassium dihydrogen phosphate, 0.5 gm.; $MgSO_4 \cdot 7H_2O$, 0.5 gm.; potassium chloride, 0.2 gm.; $FeSO_4 \cdot 7H_2O$, 0.02 gm.; $CoCl_2 \cdot 6H_2O$, 0.008 gm.; glucose, 10 gm.; yeast extract (Difco), 1.0 gm.; casamino acids (Difco), 5.0 gm.; and distilled water, 1 liter.

*Medium B:* potassium monohydrogen phosphate, 0.5 gm.; potassium dihydrogen phosphate, 0.5 gm.; $MgSO_4 \cdot 7H_2O$, 0.5 gm.; potassium chloride, 0.2 gm.; $FeSO_4 \cdot 7H_2O$, 0.02 gm.; $CoCl_2 \cdot 6H_2O$, 0.012 gm.; glucose, 10 gm.; yeast extract (Difco), 4 gm.; casamino acids (Difco), 15 gm.; and distilled water, 1 liter.

In addition to vitamin $B_{12}$ determinations final pH readings were made as well as estimations of growth by turbidity measurement with a Unicam Spectrophotometer at a wave length of 450 m$\mu$.

## Results

Table I records pH, density values, and vitamin $B_{12}$ yields (calculated as m$\mu$gm. per ml. of culture) for 70 rhizobia strains grown in Medium *A*, while average $B_{12}$ values for each species and the distribution of the yields are shown in Table II. When the species were compared, the yield of vitamin was, in general, proportional to the amount of growth; however, with strains of the same species, no close correlation was observed between vitamin yield and density. The findings indicated a surprising relationship of vitamin synthesizing capacity to the species in this closely related group of micro-

TABLE II

VITAMIN $B_{12}$ PRODUCTION BY DIFFERENT *Rhizobium* SPECIES IN CASAMINO ACIDS MEDIUM *A*

| Species | No. of strains | Optical density* (av.) | Vitamin $B_{12}$ av. yield (m$\mu$gm./ml.) | Distribution of yields (m$\mu$gm./ml.) | | | |
|---|---|---|---|---|---|---|---|
| | | | | 0-1 | 1.1-10 | 10.1-100 | >100 |
| *R. meliloti* | 12 | .204 | 410.4 | — | — | — | 12 |
| *R. japonicum* | 13 | .105 | 69.4 | — | 1 | 10 | 2 |
| *R. trifolii* | 13 | .053 | 28.6 | — | 8 | 4 | 1 |
| *R. leguminosarum* | 9 | .062 | 26.3 | — | 4 | 5 | — |
| *R. phaseoli* | 11 | .035 | 22.2 | 1 | 4 | 6 | — |
| *R. lupini* | 12 | .030 | 3.5 | 4 | 8 | — | — |

* *Culture diluted 1-30.*

organisms, and showed that *R. meliloti* was sharply distinguished from the others by its ability to produce much higher quantities of $B_{12}$. By means of a *t* test on mean yields, this organism was established as significantly different from each of the other five species at the 1% level of significance. Mean yields of the other species did not differ significantly from one another when compared in pairs. The distribution of $B_{12}$ values showed that 100% of *R. meliloti* cultures produced over 100 m$\mu$gm. $B_{12}$ per ml. while only three of the remaining 58 cultures were in that range.

A further distinction between *R. meliloti* and the other species of *Rhizobium* was the ability of all strains to grow better and produce higher quantities of vitamin $B_{12}$ in Medium *B* than in Medium *A*. On the other hand all but three of the remaining 58 strains failed to grow in this medium. As noted in Table III, the $B_{12}$ yields of the strains of *R. meliloti* ranged from 430 to 1055 m$\mu$gm. per ml. The other cultures that grew in this medium, two strains of *R. phaseoli* and one of *R. leguminosarum*, gave yields of $B_{12}$ much lower than those from any of the *R. meliloti* cultures.

*Rhizobium meliloti*, as far as the strains studied were concerned, was thus distinguished from the other species tested by its significantly higher production of vitamin $B_{12}$ and its ability to grow on Medium *B*. On neither medium was there any correlation between yield of vitamin and the effectiveness of the organism with respect to nitrogen fixation in symbiosis with the legume plant.

TABLE III

VITAMIN $B_{12}$ PRODUCTION BY *Rhizobium meliloti* IN CASAMINO ACIDS MEDIUM *B*

| Strain No. | Nitrogen-fixing capacity | Final pH | Optical density* | Vitamin $B_{12}$ (m$\mu$gm./ml.) |
|---|---|---|---|---|
| 309 | Good | 8.75 | .460 | 810 |
| 340 | Good | 8.55 | .460 | 830 |
| 524 | Good | 8.1 | .370 | 650 |
| 756 | Parasitic | 7.9 | .290 | 710 |
| 757 | Good | 8.0 | .450 | 1055 |
| 758 | Very good | 8.0 | .350 | 880 |
| 759 | Very good | 8.1 | .395 | 880 |
| 760 | Good | 8.0 | .260 | 770 |
| 785 | Good | 8.05 | .310 | 430 |
| 786 | Good | 7.8 | .345 | 490 |
| 787 | Poor | 8.2 | .385 | 935 |
| 788 | Poor | 8.0 | .320 | 510 |

* *Culture diluted 1-30.*

## Acknowledgments

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## Reference

1. BURTON, MARGARET O. and LOCHHEAD, A. G. Studies on the production of vitamin $B_{12}$-active substances by microorganisms. Can. J. Botany, 29 : 352-359. 1951.

# INHERITANCE OF RESISTANCE TO *USTILAGO NUDA* IN BARLEY[1]

By William P. Skoropad[2] and L. P. V. Johnson[3]

## Abstract

The inheritance of resistance to loose smut races Tr and Ts (a new race) was studied in several barley crosses and backcrosses in which the varieties Jet and Titan were the resistant parents. Jet is resistant to race Tr and race Ts, Titan to race Tr only. Genetical analyses were based (*a*) on a classification of $F_2$ plants according to the smut reaction of their $F_3$ progeny, and (*b*) on the reaction of selfed generation backcrosses involving recessive (susceptible) parents. The resistance of Titan and of Jet to Tr was found in each case to be due to a single, dominant gene, designated as *Un* and $Un_3$, respectively. The resistance of Jet to Ts was also found to be due to a single, dominant gene. This is probably a "new" gene and has been provisionally designated $Un_6$. Resistance was found to be determined by the genetic constitution of the developing embryo rather than by that of surrounding floral structures.

## Introduction

Loose smut of barley is caused by *Ustilago nuda* (Jens.) Rostr., a floral-infecting fungus producing a deep internal infection of the seed. The disease causes reductions in yield approximately equivalent to the percentage of smutted plants (9). The hot-water method of treating infected barley seed is effective but difficult to apply and, even under carefully controlled conditions, may cause considerable damage. Preliminary work by Tyner (13) on chemical treatments shows effective control by a Spergon solution; but, since this treatment entails 46 hr. of soaking and results in 20% mortality, it leaves much to be desired. These difficulties in controlling loose smut by treating the seed of susceptible varieties emphasize the practical importance of breeding disease-resistant varieties.

A number of investigations on the inheritance of resistance to loose smut in barley have been reported. Zeiner (15) found indications that a single gene conditioned smut reaction in each of several crosses. Resistance appeared to be dominant over susceptibility. Livingston (1) reported the presence of a single, dominant gene for resistance in Trebi and in a selection of *Hordeum deficiens*. Schaller (8) confirmed Livingston's findings concerning Trebi and demonstrated single-gene resistance in varieties Jet, Dorsett, and selection ×173–10–5–6–1. In keeping with the recommendations of Robertson, Wiebe, and Shands (3), he suggested the symbols $Un_3$, $Un_4$, and $Un_5$ for genes in Jet, Dorsett and selection ×173–10–5–6–1, respectively. He concluded that the Trebi gene was independent of the Jet and Dorsett genes. Smith (11) has recently provided a general literature review on the inheritance of resistance to loose smut in barley.

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*Contribution from the Department of Plant Science, University of Alberta, Edmonton, Alberta, based on a thesis submitted by the senior author in partial fulfillment of the requirements for the degree of Master of Science.*

[2] *Formerly Graduate Student, Department of Plant Science, University of Alberta; now Technical Officer, Dominion Laboratory of Plant Pathology, Edmonton.*

[3] *Professor of Genetics and Plant Breeding.*

Physiological specialization has been shown to occur in *U. nuda* (4, 5, 7, 12), at least 12 physiologic races having been identified. To the plant breeder such diversity of differential pathogenicity emphasizes the need for several sources of resistance.

Until 1946, barley breeders at the University of Alberta made extensive use of the almost immune variety, Titan, in their breeding program. In that year an unprecedented percentage of infection was observed in Titan, which has subsequently increased to a point where the variety must be classed as susceptible. This change in resistance of Titan to loose smut indicated the appearance of a new race of *Ustilago nuda*.

The object of the present investigation is to determine the mode of inheritance of resistance to: (*a*) the original race of loose smut to which Titan is resistant, and (*b*) the new race to which Titan is susceptible.

## Material and Methods

Since two different races of loose smut, as yet unnamed, were involved in this project it was necessary to identify them by some simple designation. In this paper the following designations are used: Tr for the race to which Titan is resistant, and Ts for the race to which Titan is susceptible.

Hybrid populations from various combinations of the spring barleys, Jet, Montcalm, Sanalta, and Titan were used in this investigation. The reaction of these varieties to the Tr and Ts races of loose smut are shown in Tables I and II, respectively.

TABLE I

REACTION OF PARENTAL VARIETIES WHEN INOCULATED ARTIFICIALLY WITH *Ustilago nuda*, RACE TR

| Variety | No. of tests* | Range of infection, % | Average infection, % |
|---|---|---|---|
| Jet | 108 | 0 | 0.0 |
| Montcalm | 117 | 55–80 | 68.3 |
| Sanalta | 98 | 65–90 | 82.6 |
| Titan | 130 | 0–4.5 | 1.1 |

* *A test denotes the progenies from three inoculated heads.*

TABLE II

REACTION OF PARENTAL VARIETIES WHEN INOCULATED ARTIFICIALLY WITH *Ustilago nuda*, RACE TS

| Variety | No. of tests* | Range of infection, % | Average infection, % |
|---|---|---|---|
| Jet | 95 | 0 | 0.0 |
| Montcalm | 85 | 65–85 | 78.0 |
| Sanalta | 92 | 70–95 | 92.8 |
| Titan | 173 | 25–50 | 45.4 |

* *A test denotes the progeny from three inoculated heads.*

Jet, in which no smutted plants have been found, is a black, naked-kerneled, two-rowed variety of Abyssinian origin. It is of no commercial importance in Canada but is valuable as a source of resistance to loose smut in breeding programs.

Titan is a white, six-rowed, smooth-awned variety which originated from the cross, Trebi × Glabron. It has a very strong straw and neck and is an important feed barley in Alberta. It is highly resistant to *Ustilago nuda*, race Tr but susceptible to *U. nuda*, race Ts.

Montcalm is a white, six-rowed, semismooth variety developed from a cross (Michigan 31604 × Common Six-rowed 4307 M.C.) × Mandscheuri 1807 M.C. Michigan 31604 is a black, smooth-awned variety, while the other two parents are of the Manchuria type. Montcalm is a malting barley widely grown in Canada. It is susceptible to both races of loose smut.

Sanalta is a white, smooth-awned, two-rowed variety which originated from the cross, Smooth Awn × Duckbill. It is highly susceptible to both races of loose smut.

The "hypodermic" method of inoculation as described by Poehlman (2) was used throughout this investigation. A rubber bulb containing an aqueous suspension of about 3% by volume of chlamydospores of *U. nuda* was attached to a hypodermic needle (20 gauge). The two lower and the two top florets of a barley head were removed because they were generally found to be late in maturing. The awns were clipped to facilitate the operation of the needle. A drop of this chlamydospore suspension was injected into each floret by piercing the lemma with the needle and squeezing the bulb slightly. Under greenhouse conditions the technique was modified in that the excess water was sucked back from the floret into the rubber bulb.

Inoculations were made at a stage extending from dehiscence to shortly after fertilization. Other workers (1, 8, 10, 6) found this to be the period at which the ovary is most susceptible to infection. The plants from the inoculated seed were grown mostly in the field. There was little difference in degree of infection between materials grown in field or greenhouse.

Inoculum of the Ts race was built up and maintained on Titan while that of Tr was maintained on Montcalm. The identity of each race of smut was frequently checked by its reaction on Titan. The inoculated seed was treated with organic mercuric dust before sowing to reduce seedling loss and increase vigor.

Hybrid material was tested in $F_1$, $F_2$, $F_3$ and selfed backcross generations. The $F_2$ does not permit a satisfactory genetical analysis since an excess of noninfected plants may result from lethal effects of infection (1, 14) or failure to obtain a phenotypic expression of all genetically susceptible plants. Classification of $F_2$ plants, based on the behavior of their progenies in $F_3$ rows, and selfed progeny tests of backcrossed plants, formed the basis for genetical interpretation.

A single smutted tiller in a plant was sufficient to classify it as susceptible. The total number of plants and the number of smutted plants were recorded and the percentage infection calculated.

In the genetic analysis, probability ($P$) values were calculated for each hypothesized ratio by the $\chi^2$ test of goodness of fit.

## Experimental Results

### I. Smut Infection in the $F_1$

It was necessary to ascertain the influence of maternal tissue on infection since the tissues through which the infecting hyphae must penetrate to the ovary are those of the maternal parent, which may differ genetically from those of the embryo to be tested for susceptibility. This problem was investigated by observing the smut reaction of $F_1$ plants in reciprocal crosses.

The smut reactions in the $F_1$ of reciprocated crosses artificially inoculated with races Tr and Ts are shown in Table III.

TABLE III

Infection of $F_1$ barley hybrids inoculated with race Tr and with race Ts

| Hybrid* | Tr inoculation | | Ts inoculation | |
|---|---|---|---|---|
| | No. of plants | Infection, % | No. of plants | Infection, % |
| Sanalta × Jet | 55 | 0.0 | 66 | 0.0 |
| Jet × Sanalta | 42 | 0.0 | 188 | 0.0 |
| Sanalta × Titan | 84 | 2.4 | 88 | 56.8 |
| Titan × Sanalta | 106 | 1.9 | 123 | 55.3 |
| Montcalm × Jet | 163 | 0.0 | 156 | 0.0 |
| Jet × Montcalm | 87 | 0.0 | 96 | 0.0 |
| Montcalm × Titan | 93 | 1.1 | 74 | 52.7 |
| Titan × Montcalm | 84 | 0.0 | 108 | 55.6 |
| Titan × Jet | 55 | 0.0 | 177 | 0.0 |

* *Female parent given first.*

The degree of infection in reciprocal crosses shows no influence of the maternal tissue. The absence of infection in the $F_1$ of all crosses involving Jet as the male parent must be attributed to the heterozygous embryo, as the floral tissue of Sanalta and Montcalm was susceptible. Resistance to infection must, therefore, be determined by the genetic constitution of the developing embryo rather than that of the surrounding floral tissue. The gene(s) for resistance in Jet must be completely dominant since immunity to loose smut is maintained even in the heterozygous condition.

The degree of Tr infection in the $F_1$ hybrids satisfies genetic expectation (assuming resistance to be dominant) on the basis of infection data given for resistant parents, Jet and Titan, in Table I.

The observation that $F_1$ hybrids involving Titan show higher infection by Ts than that of Titan itself (Table II) may be attributed to incomplete dominance of the Titan gene or to interaction with Sanalta and Montcalm genes.

## II. Inheritance of Resistance

*1. Observations in the $F_1$*

The degree of infection in $F_1$ hybrids as shown in Table III demonstrates that resistance of Jet and of Titan is transmitted as a dominant character. Since no smutted plants were observed in hybrids involving Jet, it may be concluded that resistance is completely dominant in this variety. Hybrids involving Titan and inoculated with race Tr, show average infections which correspond very closely to the average infection of Titan (Table I), indicating that the resistance of Titan is also completely dominant.

*2. $F_2$ and $F_3$ Data on Resistance to Race Tr*

*(a) Reaction to Race Tr*

(Since two different races of loose smut were used in this study it will be necessary to discuss results of each set of inoculations separately, and hence they are listed under separate headings for the $F_2$ and $F_3$ generations.)

Infection data for the $F_2$ generation are given in Table IV. The proportion of infected plants shown in Table IV suggests a dihybrid ratio. However, it must be borne in mind that the susceptible parents, Montcalm and Sanalta, did not develop 100% infection, and that the infection distribution of the hybrids will be related to that of the parents.

Since it is not possible to determine the genetics of resistance directly from the $F_2$ population, the genetical interpretation must come from observations on the $F_3$ and selfed backcross generations.

*(b) Inheritance of Reaction to Tr in Jet Hybrids*

Data relating to the genetic analyses of Tr smut reaction in the Jet crosses and backcrosses are given in the first parts of Tables V, VI, VII, and VIII.

Smut infection data obtained from $F_3$ and backcross lines, classified in 5% class intervals, are given in Table V. The infection distributions thus obtained are bimodal (trimodal if the 0% class is considered separately) which permits the separation of phenotypic classes for purposes of genetic analysis. The point of separation is indicated by parenthetic marks within each distribution.

TABLE IV

Infection of $F_2$ barley hybrids inoculated with race Tr and with race Ts

| Hybrid* | Tr inoculation | | Ts inoculation | |
|---|---|---|---|---|
| | No. of plants | Infection, % | No. of plants | Infection, % |
| Sanalta × Jet | 496 | 4.4 | 602 | 8.2 |
| Montcalm × Jet | 689 | 3.9 | 287 | 8.0 |
| Sanalta × Titan | 581 | 11.2 | 868 | 73.2 |
| Montcalm × Titan | 430 | 7.9 | 857 | 58.8 |
| Titan × Jet | 260 | 0.0 | 477 | 4.2 |

* *These hybrids (excepting Titan × Jet, Tr, and Montcalm × Jet, Ts) were studied in reciprocal crosses. No significant differences in infection were observed between reciprocals.*

## TABLE V

DISTRIBUTION OF $F_2$ AND BACKCROSS PROGENIES FOR LOOSE SMUT INFECTION BY PERCENTAGE CLASSES FOR CROSSES ARTIFICIALLY INOCULATED WITH RACE TR

| Crosses | Total no. of rows | Percentage classes | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 0.1–5 | 5–10 | 10–15 | 15–20 | 20–25 | 25–30 | 30–35 | 35–40 | 40–45 | 45–50 | 50–55 | 55–60 | 60–65 | 65–70 | 70–75 | 75–80 | 80–85 | 85–90 | 90–95 | 95–100 |
| Sanalta × Jet | 204 | 58 | 18 | 28 | 30 | 14 | 11 | 2 | 0 | 0 | )0(* | 0 | 0 | 2 | 1 | 6 | 8 | 5 | 9 | 1 | 7 | 4 |
| Sanalta × $F_1$ (Sanalta × Jet) | 84 | | 25 | 4 | 3 | 1 | 1 | 1 | 1 | 0 | )0( | 0 | 2 | 1 | 3 | 7 | 3 | 6 | 14 | 8 | 4 | 0 |
| Montcalm × Jet | 192 | 55 | 32 | 21 | 24 | 6 | 13 | 0 | 2 | )0( | 1 | 2 | 1 | 3 | 9 | 20 | 3 | 0 | 0 | 0 | 0 | 0 |
| Montcalm × $F_1$ (Montcalm × Jet) | 116 | | 46 | 6 | 1 | 2 | 3 | 2 | 1 | 2 | )0( | 0 | 3 | 3 | 31 | 2 | 11 | 0 | 1 | 0 | 0 | 0 |
| Sanalta × Titan | 216 | 38 | 47 | 31 | 19 | 10 | 5 | 0 | )0( | 2 | 1 | 0 | 7 | 15 | 29 | 10 | 2 | 0 | 0 | 0 | 0 | 0 |
| Sanalta × $F_1$ (Sanalta × Titan) | 64 | 0 | 0 | 12 | 2 | 8 | 2 | 1 | 1 | )0( | 3 | 2 | 5 | 18 | 4 | 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| Montcalm × Titan | 166 | 29 | 33 | 26 | 18 | 6 | 10 | 3 | 5 | 1) | (1 | 5 | 9 | 17 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Titan × Montcalm | 184 | 41 | 42 | 35 | 4 | 4 | 4 | 5 | 3 | 1 | 2 | 1) | (4 | 26 | 8 | 1 | 1 | 2 | 0 | 0 | 0 | 0 |

* *Parenthetic marks indicate division of the distribution into phenotypic classes used in the genetical analysis of Tables VI, VII, and VIII.*

Table VI presents tests of goodness of fit to the 3:1 ratio for each of the infection distributions involving Jet hybrids. The $P$ values obtained indicate an acceptable fit in each case. It may therefore be assumed that a single gene controls smut reaction, resistance being dominant.

Since Jet is completely resistant to Tr, it may be assumed that all $F_3$ lines falling into the 0% class must have been derived from homozygous resistant $F_2$ plants; and, since the $F_3$ lines falling into the high infection classes coincide

TABLE VI

TESTS OF GOODNESS OF FIT OF THE INFECTION DATA (TR) OF TABLE V TO THE MONOHYBRID RATIO, 3:1

| Hybrid | Resistant | Susceptible | $P$ |
|---|---|---|---|
| Sanalta × Jet | 161 | 43 | 0.19 |
| Montcalm × Jet | 153 | 39 | 0.15 |
| Sanalta × Titan | 150 | 66 | 0.07 |
| Montcalm × Titan | 131 | 35 | 0.25 |
| Titan × Montcalm | 142 | 42 | 0.50 |

TABLE VII

TESTS OF GOODNESS OF FIT OF THE INFECTION DATA (TR) OF TABLE V TO THE RATIO, 1 HOMOZYGOUS RESISTANT : 2 HETEROZYGOUS : 1 HOMOZYGOUS, WHICH SERVES TO CONFIRM THE MONOHYDRID RATIO HYPOTHESIZED IN TABLE VI

| Hybrid | Hom. resist. | Het. | Hom. susc. | $P$ |
|---|---|---|---|---|
| Sanalta × Jet | 58 | 103 | 43 | 0.34 |
| Montcalm × Jet | 55 | 98 | 39 | 0.26 |
| Sanalta × Titan | 38 | 112 | 66 | 0.12 |
| Montcalm × Titan | 29 | 102 | 35 | 0.03 |
| Titan × Montcalm | 41 | 101 | 42 | 0.48 |

TABLE VIII

TESTS OF GOODNESS OF FIT OF THE INFECTION (BACKCROSS) DATA (TR) OF TABLE V TO THE RATIO, 1 HETEROZYGOUS : 1 HOMOZYGOUS SUSCEPTIBLE, WHICH SERVES TO CONFIRM THE MONOHYBRID RATIO HYPOTHESIZED IN TABLE VI

| Hybrid | Het. | Hom. susc. | $P$ |
|---|---|---|---|
| Sanalta × $F_1$ (Sanalta × Jet) | 36 | 48 | 0.19 |
| Montcalm × $F_1$ (Montcalm × Jet) | 65 | 51 | 0.20 |
| Sanalta × $F_1$ (Sanalta × Titan) | 26 | 38 | 0.33 |

very well with the infection ranges of the susceptible parents, Sanalta and Montcalm (Table I), it may be assumed that these lines are derived from homozygous susceptible $F_2$ plants. Further, it may be assumed that $F_3$ lines falling in the intermediate classes were produced from heterozygous $F_2$ plants. These assumptions permit division of the distributions into homozygous resistant, heterozygous, and homozygous susceptible groups which should theoretically, occur in a 1:2:1 ratio. This forms the basis for the genetic analysis presented in Table VII.

Table VII gives tests of goodness of fit to a 1:2:1 ratio for each of the smut reaction distributions of Jet hybrids. Highly acceptable fits are obtained which substantiate the single gene hypothesis assumed for the previous test.

The assumed hypothesis may be tested still further by recourse to data of the selfed generation from backcrosses. These data are given in Table V and are subjected to goodness of fit tests in Table VIII. The fits to the 1:1 ratio are quite acceptable.

We may conclude from the results of these triple analyses that resistance to Tr smut in the crosses Sanalta × Jet and Montcalm × Jet is, in each case, controlled by a single, dominant gene.

(*c*) *Inheritance of Reaction to Tr in Titan Hybrids*

Data relating to the genetic analysis of Tr smut in Titan crosses and backcrosses are given in the last parts of Tables V, VI, VII, and VIII.

The analysis of smut reaction in the Titan crosses follows exactly the pattern already used for the Jet hybrids. It will not be necessary, therefore, to repeat the experimental considerations of the previous section.

The goodness of fit tests presented in Table VI show an acceptable fit to the 3:1 ratio for each of the Titan crosses, though Sanalta × Jet approaches the borderline of nonacceptability.

Acceptable fits to the 1:2:1 ratio are shown in Table VII, except in the case of Montcalm × Titan. The latter case may be disregarded, however, since the reciprocal, Titan × Montcalm, shows an excellent fit.

Data on smut reaction in the selfed backcross of Sanalta × $F_1$ (Sanalta × Titan), given in Table VIII, show a good fit to the 1:1 ratio.

All three analyses of reaction to Tr smut in the Titan hybrids are in agreement in showing resistance to be inherited on the basis of a single, dominant gene.

*3. $F_2$ and $F_3$ Data on Resistance to Race Ts*

(*a*) *Reaction to race Ts*

Infection data for the $F_2$ hybrids, inoculated with race Ts, are given in Table IV.

Comparison of the infection rate of $F_2$ Jet hybrids when inoculated with Tr or with Ts showed a consistently higher rate of infection from the Ts inoculum. Jet has been found to be completely resistant to both races of loose smut (Tables I and II) and, therefore, the above results may be attributed to the greater susceptibility of Sanalta, Montcalm, and Titan to Ts.

## TABLE IX

DISTRIBUTION OF $F_2$ AND BACKCROSS PROGENIES FOR LOOSE SMUT INFECTION BY PERCENTAGE CLASSES FOR CROSSES INDICATED. ARTIFICIALLY INOCULATED WITH RACE Ts

| Crosses | No. of lines | Percentage classes | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 0.1–5 | 5–10 | 10–15 | 15–20 | 20–25 | 25–30 | 30–35 | 35–40 | 40–45 | 45–50 | 50–55 | 55–60 | 60–65 | 65–70 | 70–75 | 75–80 | 80–85 | 85–90 | 90–95 | 95–100 |
| Sanalta × Jet | 184 | 52 | 36 | 16 | 14 | 8 | 7 | 5 | 3 | 1 | 1 | 3 | 1 | 0) | (*0 | 2 | 0 | 3 | 11 | 18 | 1 | 2 |
| Sanalta × $F_1$ (Sanalta × Jet) | 108 | 0 | 36 | 16 | 3 | 0 | 2 | 3 | 1 | 0 | 0 | )0( | 0 | 0 | 8 | 3 | 3 | 10 | 22 | 0 | 1 | |
| Montcalm × Jet | 130 | 42 | 0 | 1 | 8 | 1 | 14 | 20 | 2 | 1 | 1 | )0( | 1 | 3 | 2 | 5 | 9 | 17 | 0 | 3 | 0 | 0 |
| Montcalm × $F_1$ (Montcalm × Jet) | 98 | 0 | 0 | 1 | 29 | 17 | 5 | 3 | 1 | 1 | 2 | )0( | 1 | 2 | 4 | 8 | 5 | 18 | 1 | 0 | 0 | 0 |
| Titan × Jet | 84 | 66 | )0( | 1 | 2 | 0 | 0 | 8 | 4 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* *Parenthetic marks indicate division of the distribution into phenotypic classes used in the genetical analyses of Tables X, XI, and XII.*

$F_2$ hybrids involving Titan and Sanalta as well as Titan and Montcalm present a different picture. The average infection of the $F_2$ progenies of the cross Sanalta × Titan is 73.16% (Table VII) which approaches the infection rate of Sanalta (Table II). Titan appears to contribute only a slight degree of resistance to its $F_2$ hybrids and thus confirms the statement made regarding its $F_1$ hybrids: that Titan carries only weak resistance to Ts.

For reasons given previously, genetic analyses were not attempted directly from the $F_2$ populations.

(*b*) *Inheritance of Reaction to Race Ts in Jet Hybrids*

The genetic analyses in this section follow the method used previously in connection with race Tr.

Data on infection by race Ts obtained from $F_3$ and backcross lines and classified in 5% class intervals are given in Table IX. The infection distributions are bimodal, or trimodal if the 0% class is taken separately, which permits division into phenotypic classes. The point of this division is indicated by parenthetic marks within each distribution.

Table X presents tests of goodness of fit to the 3:1 ratio for each of the Jet crosses. An acceptable fit is obtained in each case, leading to the assumption of a single, dominant gene for resistance.

TABLE X

TESTS OF GOODNESS OF FIT OF THE INFECTION DATA (Ts) OF TABLE IX TO THE MONOHYBRID RATIO, 3:1

| Hybrid | Resistant | Susceptible | *P* |
|---|---|---|---|
| Sanalta × Jet | 147 | 37 | 0.13 |
| Montcalm × Jet | 90 | 40 | 0.13 |
| Titan × Jet | 66 | 18 | 0.46 |

TABLE XI

TESTS OF GOODNESS OF FIT OF THE INFECTION DATA (Ts) OF TABLE IX TO THE RATIO, 1 HOMOZYGOUS RESISTANT : 2 HETEROZYGOUS : 1 HOMOZYGOUS SUSCEPTIBLE, WHICH SERVES TO CONFIRM THE MONOHYBRID RATIO HYPOTHESIZED IN TABLE X

| Hybrid | Hom. resist. | Het. | Hom. susc. | *P* |
|---|---|---|---|---|
| Sanalta × Jet | 52 | 95 | 37 | 0.27 |
| Montcalm × Jet | 42 | 48 | 40 | 0.03 |

The further analyses in Table XI show a good fit of the 1:2:1 ratio to Sanalta × Jet data, but the fit to the Montcalm × Jet data is such that it would occur only about three times in 100 trials on the basis of sampling chance.

However, with due consideration to the preponderance of favorable statistical evidence in related tests, this need not be taken as an invalidation of the hypothesis.

Data from the selfed backcrosses are given in Table XII. Here acceptable fits to the 1:1 ratio are obtained for both backcrosses.

The three analyses of Ts smut reaction in Jet hybrids are considered to provide a reasonably good basis for the conclusion that resistance to race Ts is controlled by a single, dominant gene.

TABLE XII

TESTS OF GOODNESS OF FIT OF THE INFECTION (BACKCROSS) DATA (Ts) OF TABLE IX TO THE RATIO, 1 HETEROYZGOUS : 1 HOMOZYGOUS SUSCEPTIBLE, WHICH SERVES TO CONFIRM THE MONOHYBRID RATIO HYPOTHESIZED IN TABLE X

| Hybrid | Het. | Hom. susc. | *P* |
|---|---|---|---|
| Sanalta × $F_1$ (Sanalta × Jet) | 61 | 47 | 0.22 |
| Montcalm × $F_1$ (Montcalm × Jet) | 59 | 39 | 0.15 |

## Discussion

The experimental results have shown that the resistance (incomplete) of Titan to race Tr is due to a single, dominant gene. This gene is presumably the same as that found by other workers (1, 8) in Trebi and designated as *Un* by Robertson *et al.* (3). Titan and Trebi are related and appear to react identically to loose smut.

The resistance of Jet to race Tr is also due to a single, dominant gene. This gene is probably not *Un* since Schaller (8), working with a race of loose smut presumably identical with Tr, found the resistance of Jet to be due to a gene, designated as $Un_3$, distinct from *Un*.

Jet, unlike Titan, is resistant to race Ts. This resistance is clearly due to a single, dominant gene. Since the inheritance of reaction to Ts has not, so far as is known, been studied before, this gene is probably not among those previously identified and named. Complying with the recommendations of Robertson *et al.* (3) this "new" gene is given the next available designation, $Un_6$.

The results would seem to prove that a single dominant gene conditions smut resistance in each of the following three cases: (*a*) resistance of Titan to Tr, (*b*) resistance of Jet to Tr, and (*c*) resistance of Jet to Ts. There is no proof, however, that three distinct genes are involved. For example, it could be assumed that Jet possesses the genes *Un* and $Un_6$, and that complementary or supplementary interaction between these genes produces the action attributed to $Un_3$. This would mean that there is no individual gene $Un_3$. Similarly it might be demonstrated how the presence and interaction of *Un* and $Un_3$ obviates the necessity of assuming the presence of $Un_6$.

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# WINTER CROWN ROT OR SNOW MOLD OF ALFALFA, CLOVERS, AND GRASSES IN ALBERTA

## II. FIELD STUDIES ON HOST AND VARIETAL RESISTANCE AND OTHER FACTORS RELATED TO CONTROL[1]

By M. W. Cormack[2]


## Abstract

Many overwintering cultivated and native plants proved susceptible to the low-temperature basidiomycete which causes winter crown rot or snow mold of forage legumes and grasses in Alberta. Tests were made by inoculation in the late fall, or by planting in infested land. In the legumes, high resistance was found only in *Medicago falcata* and a Siberian strain of *Trifolium pratense*. *T. hybridum*, *Melilotus* spp., and the commonly grown varieties of alfalfa were particularly susceptible. *Bromus* spp. and *Agropyron* spp. proved highly resistant. Resistance was moderate in *Elymus* spp., *Poa pratensis*, and *Festuca elatior*; and slight in *F. rubra* and *Phleum pratense*. *Agrostis alba*, winter wheat, and winter rye were highly susceptible. In tests on horticultural plants, rhubarb and chives appeared to be immune; raspberry, peony, and columbine were resistant; and strawberry, parsnip, iris, and tulip were highly susceptible. A wide range of native plants and weeds suffered severe damage, and only *Thalictrum* spp. proved highly resistant. Shrubs and trees were not attacked.

Following severe damage, crop rotation proved to be essential for the control of winter crown rot. The pathogen did not persist in the soil to any extent for longer than two to three years in land which was summer-fallowed or planted to annual crops or resistant grasses. Brome and other resistant grasses proved helpful in maintaining forage stands. Fertilizer treatments did not reduce the damage. Uncut tops and debris favored spread of the disease in alfalfa stands.


## Introduction

As previously reported (3), winter crown rot or snow mold, caused by an unidentified low-temperature basidiomycete, is often of major importance in alfalfa and other overwintering crops in the central and northern areas of Alberta and Saskatchewan. The best method of controlling this disease would be to grow resistant varieties. Such varieties, however, are not yet available and may require considerable time for development. In the meantime, other methods are being sought for controlling or reducing the damage.

The main purpose of the present study was to obtain additional information on the relative resistance of crops and varieties which would aid in the selection of breeding material and in the planning of suitable crop rotations. The host range of the pathogen was further studied in inoculation tests on various overwintering fruits, vegetables, and ornamental plants. Observations and tests were also made on various wild species in an attempt to determine their possible role in harboring the pathogen.

The ability of the pathogen to survive under various conditions was studied in relation to control by crop rotation. Data were also obtained on the influence of forage mixtures, fertilizers, cutting practices, and other factors on degree of infection.

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## Host and Variety Tests

The relative resistance of different forage crops and other overwintering plants to the winter crown rot pathogen was determined by various methods of inoculation in the late fall, or by planting in infested land. In studying host reaction, a small quantity of inoculum grown on a soil–cornmeal medium was placed in the surface soil near the crowns of the plants. Varietal differences were often obscured, however, by the severe infection which usually resulted from this method. In some experiments, the distance of spread of killing was measured from marked points of inoculation in plots or rows (3). Most varietal tests were made in infested land in which a stand of susceptible alfalfa had been killed out by inoculation. This was done by broadcasting inoculum among the alfalfa plants in the fall at a rate of 25 cc. per sq. ft., or by applying it along the rows near the crowns of the plant with a V-belt seeder. Nearly all plants in stands thus treated were dead the following spring (Fig. 1). The plant remains were thoroughly mixed through the upper soil with a rototiller cultivator to obtain uniformly infested land. Crops planted in this land grew normally during the first season. The percentage of survival from winter infection was determined in the following spring (Fig. 2). Data obtained by these methods on the comparative reaction of commonly grown crops are summarized in Table I.

TABLE I

COMPARATIVE RESISTANCE OF FORAGE CROPS AND WINTER CEREALS TO THE WINTER CROWN ROT PATHOGEN, AS DETERMINED IN THE FIELD BY DIRECT INOCULATION, SPREAD OF KILLING FROM POINTS OF INOCULATION, AND SURVIVAL IN INFESTED LAND. (AVERAGE RESULTS OF FOUR EXPERIMENTS)

| Crop | Variety | Reaction to inoculation | Spread of killing, in. | Survival in infested land, % |
|---|---|---|---|---|
| Alfalfa | Grimm | Highly susc. | 7.0 | 9 |
| Alfalfa | *M. falcata* | Highly resist. | 4.8 | 87 |
| Sweet clover | Arctic | Highly susc. | 7.1 | 4 |
| Red clover | Altaswede | Susc. | 6.3 | 26 |
| Red clover | Siberian | Mod. resist. | 5.2 | 68 |
| Alsike clover | Commercial | Highly susc. | 6.8 | 10 |
| Ladino clover | Commercial | Susc. | 4.8 | 21 |
| White clover | Commercial | Susc. | 4.9 | 20 |
| Brome grass | Parkland | Highly resist. | 0 | 100 |
| Crested wheat grass | Fairway | Highly resist. | 2.0 | 83 |
| Slender wheat grass | Commercial | Highly resist. | 2.2 | 85 |
| Kentucky blue grass | Commercial | Mod. resist. | 2.1 | 76 |
| Meadow fescue | Commercial | Mod. resist. | 2.4 | 63 |
| Creeping red fescue | Olds | Slightly resist. | 3.7 | 36 |
| Timothy | Commercial | Slightly resist. | 3.2 | 52 |
| Red top | Commercial | Highly susc. | 5.8 | 27 |
| Winter rye | Commercial | Highly susc. | 3.4 | 12 |
| Winter wheat | Kharkov | Highly susc. | 4.4 | 3 |

## Forage Legumes

### *Alfalfa*

In previous tests with alfalfa (2), several commonly grown varieties proved highly susceptible. Further information was obtained in experiments conducted for several years in uniformly infested land. The relative resistance of the species and varieties studied was as follows:

Highly resistant (85–90% survival)—*Medicago falcata* L.

Moderately resistant (40–60% survival)—Rhizoma

Slightly resistant (10–25% survival)—Cossack, Viking

Highly susceptible (0–10% survival)—Buffalo, Canauto, Ferax, Grimm, Hardistan, Ladak, *M. glutinosa*, Ontario variegated, Orestan, Ranger, Turkistan, Utah Common.

The results of all tests (Table I) show that *M. falcata* is highly resistant, as compared to Grimm and other commonly grown varieties. Certain strains of this species appear to be nearly immune since they have survived repeated inoculations under particularly severe conditions. The moderate to slight resistance of the varieties Rhizoma, Cossack, and Viking was probably derived from the *M. falcata* in their parentage. Stands of these varieties may last for a year or two longer than those of Grimm, unless conditions are particularly favorable for disease development. All other varieties tested proved highly susceptible and were completely destroyed in some seasons (Fig. 3).

### *Sweet Clover*

Sweet clover proved highly susceptible to the winter crown rot pathogen (Table I). Several tests were made on the following species and varieties: *Melilotus alba* Desr.—Alpha, Arctic, Brandon Dwarf, Common White, Grundy County, Wisconsin D236; *M. officinalis* (L.) Lam.—Albotrea, Aura, Common Yellow, Erector; *M. suaveolens* Ledeb.—Redfield Yellow. There was little or no survival in any of these varieties following inoculation or planting in infested land. Also, no significant varietal differences were found in the spread of killing in blocks or rows.

### *Red Clover*

Most varieties of red clover (*Trifolium pratense* L.) tested were fairly susceptible. The average survival of Altaswede, the variety most commonly grown in Alberta, was 26% when planted in infested land. A slightly lower survival of 20 to 25% occurred in Dollard, Graham, Mammoth, and Manhardy. The variety Siberian, however, proved moderately to highly resistant, with an average survival of 68% (Table I). This resistance was particularly evident in one season when all varieties except Siberian were completely destroyed (Fig. 4). Unfortunately, this variety is highly susceptible to northern anthracnose, caused by *Kabatiella caulivora* (Kirch.) Karak, to which Altaswede is relatively resistant.

*Other Clovers*

Alsike clover (*Trifolium hybridum* L.) proved extremely susceptible (Figs. 2, 4), and suffered more damage than red clover in all tests (Table I). As previously reported (3), alsike clover is also often severely damaged under natural conditions. The variety Alon, which has been tested on a limited scale, is apparently as susceptible as the ordinary commercial strains. Ladino and white clover (*T. repens* L.) were slightly less susceptible than alsike clover (Table I).

Other hardy legumes which suffered severe damage when planted in infested land were bird's-foot trefoil (*Lotus corniculatus* L.), strawberry clover (*Trifolium fragiferum* L.), and three strains of sainfoin (*Onobrychis viciaefolia* Scop.).

## Grasses and Winter Cereals

Brome grass (*Bromus inermis* Leyss.) proved extremely resistant or almost immune to the winter crown rot pathogen in all tests (Table I). No killing occurred when it was planted in highly infested land (Figs. 2, 5), and the only damage noted was a slight lesioning and weakening of the plants under extreme conditions of direct inoculation. Common brome, the variety Parkland, and several selected strains, supplied by the Dominion Forage Crops Laboratory at Saskatoon, proved equally resistant. The results of limited tests with *B. anomalus* Rupr., *B. ciliatus* L., *B. erectus* Huds., and *B. pumpellianus* Scribn. indicate that these species also possess a high degree of resistance.

Most species of *Agropyron* were relatively resistant. In several tests in infested land, there was an average survival of from 80 to 100% in *A. cristatum* (L.) Gaertn., *A. desertorum* (Fisch.) Schult., *A. intermedium* (Host.) Beauv., *A. sibiricum* (Willd.) Beauv., and *A. trachycaulum* (Link) Malte. This reduction in stand did not have a serious effect, since the survivors thickened up and made good growth later in the season (Fig. 6). The commonly grown crested wheat grass (*A. cristatum*) and slender wheat grass (*A. trachycaulum*) were equally resistant in all tests (Table I). The average survival in seven selected strains of crested wheat grass ranged from 90 to 100%. Tall wheat grass (*A. elongatum* (Host.) Beauv.) proved moderately resistant, with an average survival of 50%. With this species, the average spread of infection along the row was 6.5 in., as compared to 2.0 in. in *A. cristatum*.

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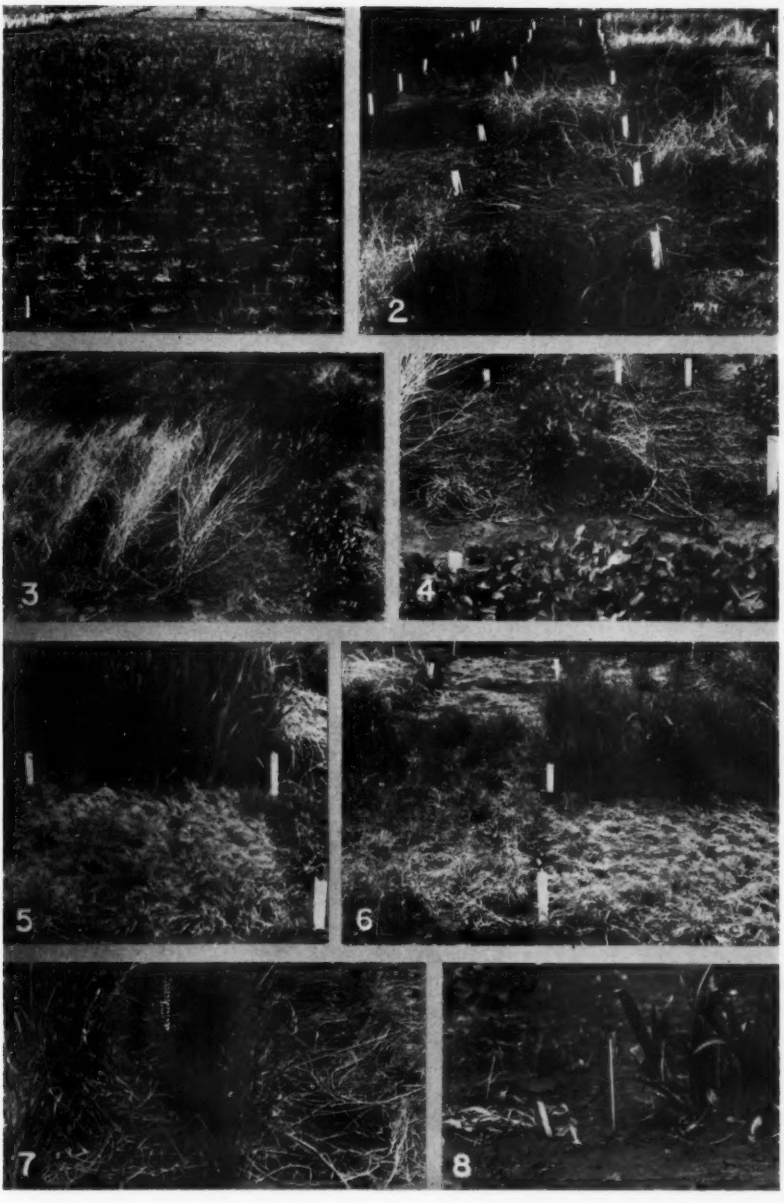
Fig. 1. Land infested with the winter crown rot pathogen obtained by killing a stand of alfalfa by fall inoculation. (Note mycelium on dead plants in rows.)

Figs. 2–7. Survival of legumes and grasses in the spring following planting in infested land. Fig. 2. General view of portion of land shown in Fig. 1; resistant brome grass in foreground, with plot of dead alsike clover behind. Fig. 3. Complete killing of several varieties of alfalfa; Altaswede and Siberian red clover at right. Fig. 4. Rows, left to right: Altaswede and Siberian red clover, alsike clover, ladino (healthy controls in foreground). Fig. 5. Dead plot of red top, with undamaged brome grass behind. Fig. 6. Plots, left to right: timothy and red top, with meadow fescue and crested wheat grass behind. Fig. 7. Rows, left to right: timothy, Kentucky blue grass, creeping red fescue.

Fig. 8. Iris plants, inoculated (left), noninoculated (right).

PLATE I



*Elymus* spp. proved moderately to highly resistant. The average survival in infested land was as follows: *E. canadensis* L.—75%, *E. junceus* Fisch.—75%, *E. virginicus* L.—87%, *E. virginicus* var. *submuticus* Hook.—92%, and *E. macounii* Vasey.—95%. The commonly occurring wild rye (*E. macounii*) appears to be particularly resistant.

Kentucky blue grass (*Poa pratensis* L.) showed moderate to high resistance in most tests (Table I and Fig. 7). It has suffered damage, however, in lawns and meadows when conditions were particularly favorable for disease development. In tests with other species, the average survival was as follows: *P. ampla* Merr.—54%, *P. bulbosa* L.—14%, and *P. trivialis* L.—15%.

The fescues varied in degree of resistance. As shown in Table I, meadow fescue (*Festuca elatior* L.) (Fig. 6) was moderately resistant, and had a consistently higher rate of survival than creeping red fescue (*F. rubra* L.) (Fig. 7). The latter often suffers severe damage in lawns and greens in the northern and central areas (3). Results of limited tests with the less hardy chewings fescue (*F. rubra* L. var. *commutata* Gaud.) and sheep fescue (*F. ovina* L.) indicate that these species are also susceptible.

Timothy (*Phleum pratense* L.) proved moderately susceptible in most tests (Table I and Figs. 6, 7), and was frequently damaged under natural conditions (3). The varieties Swallow, Boon, and Dura reacted similarly to the commercial strains.

Red top (*Agrostis alba* L.) was the most susceptible of the grasses tested. This grass did not survive artificial inoculation and usually had a high mortality when planted in infested land (Figs. 5, 6). As previously reported (3), red top also suffers damage under natural conditions.

The fall sown cereals proved as susceptible as any of the grasses and suffered severe damage in all tests (Table I). Winter rye had a slight but consistently higher degree of survival than winter wheat. The winter wheat varieties Kharkov, Minhardi, Turkey, and Yogo were all equally susceptible.

## Fruits and Vegetables

Various overwintering fruits and vegetables were tested as possible hosts for the winter crown rot pathogen. Plants of strawberry, raspberry, parsnip, rhubarb, and chives were inoculated *in situ* in the late fall. Roots of carrots and other vegetables which require storage in this climate were inoculated and, with controls, were buried about 18 in. below ground in November. This was necessary because previous results (3) had shown that plants require exposure to a temperature at or near freezing before they become susceptible to infection by the pathogen. The roots were removed and examined in mid-January before the surrounding soil was completely frozen.

Strawberries succumbed completely when inoculum was scattered thinly among the plants, while adjacent blocks of noninoculated control plants were not injured. These results suggest that the pathogen may be a factor in the so-called winterkilling of strawberries in some localities. Raspberries proved

highly resistant. The only evidence of damage in inoculated plants was a superficial lesioning at the base of the canes, resulting in a slight retardation of growth in the spring.

Rhubarb and chives appear to be immune from attack by the winter crown rot pathogen. No lesioning or other damage occurred even when the plants were repeatedly inoculated in successive seasons and under conditions which produced severe damage in other plants.

The root vegetables proved susceptible in varying degrees. Parsnips were invariably killed and partially rotted as the result of placing inoculum near the crowns in the late fall. Natural infection of this vegetable was previously reported (3). Inoculated carrots were extensively rotted while the controls remained sound. Onions and turnips were less severely damaged.

### Ornamental Plants

Since natural infection occurs in various cultivated perennial plants (3), certain herbaceous perennials, roses, and tulip bulbs were included in the inoculation tests. Inoculum was placed in contact with the crown or just above the bulb or rhizome of each plant. On the basis of average results, the plants tested were classed as follows:

Highly resistant—Columbine (*Aquilegia vulgaris* L.)

Moderately resistant—Peony (*Paeonia* sp.)

Slightly resistant—Rose (Hybrid tea), Iris (*Iris germanica* L.), Lily of the valley (*Convallaria majalis* L.), *Viola cornuta* L. (Jersey Gem).

Highly susceptible—*Baby's-breath* (*Gypsophila paniculata* L.), Yarrow (*Achillea ptarmica* L.), Chinese delphinium (*Delphinium chinense* Fisch.), Virgin's-bower (*Clematis heracleifolia* DC.), Tulip (Darwin).

The common columbine appears to be the most resistant of the ornamentals tested. Crown lesioning was slight or absent and the plants suffered no visible damage. Inoculated peonies were three weeks late in emerging from the ground, as compared to the controls, but made fair growth later in the season. When the same plants were re-inoculated the following winter they were severely weakened and did not bloom. Hybrid tea roses were damaged through killing or weakening of the buds, but the canes were not extensively lesioned. Other plants which showed slight resistance were viola, lily of the valley, and iris. Inoculated iris plants were often seriously weakened through rotting of the crowns (Fig. 8). Natural infection was previously reported in iris (3).

The plants classed as highly susceptible were killed as a result of inoculation. In all cases, the damage resulted from rotting of the crowns or upper portions of the roots. Inoculated tulip bulbs were completely rotted, while the control bulbs in adjacent rows remained sound.

Several kinds of shrubs and trees were tested by placing inoculum slightly below ground in contact with the wounded surface of the wood. When notes were taken the following spring, there was slight superficial lesioning but no obvious damage in any of the following species tested: *Amelanchier alnifolia*

Nutt., *Populus tacamahacca* Mill., *Populus tremuloides* Michx., *Prunus melanocarpa* (A. Nels.) Rydb., *Salix* spp., *Symphoricarpos occidentalis* Hook. The failure of the pathogen to attack trees and shrubs indicates that it is primarily a parasite of herbaceous plants.

### Native Plants and Weeds

Considerable information was obtained on the relative susceptibility of various native plants and weeds by identifying the species killed as the result of natural infection in snow mold patches (3), and by inoculation. Plants inoculated *in situ* in the late fall were staked and compared with adjacent noninoculated control plants in the following spring. On the basis of data obtained by these methods the species studied were classed as follows:

Highly resistant—*Thalictrum* spp.

Moderately resistant—*Achillea lanulosa* Nutt., *Chamaenerion spicatum* (Lam.) S. F. Gray, *Heracleum lanatum* Michx., *Rumex crispus* L., *Solidago* sp.

Slightly resistant—*Agropyron repens* (L.) Beauv., *Apocynum androsaemifolium* L., *Artemisia gnaphalodes* Nutt., *Aster laevis* L., *Aster multiflorus* Ait., *Vicia americana* Muhl., *Viola* sp.

Highly susceptible—*Argentina anserina* (L.) Rydb., *Cirsium arvense* (L.) Scop., *Galium boreale* L., *Lactuca pulchella* (Pursh) DC., *Lathyrus ochroleucus* Hook., *Sonchus arvensis* L., *Taraxacum* spp., *Urtica gracilis* Ait.

Meadow rue (*Thalictrum* spp.) was the only native plant which proved highly resistant, although several others showed a moderate to slight degree of resistance. Some plants were difficult to classify since their apparent resistance was mainly due to escape by means of stolons or rhizomes. This was the case with quack grass (*Agropyron repens*) which suffered severe damage in the vicinity of the inoculum but soon recovered by producing new stolons. Other weed plants which often regenerated following apparent killing were Canada thistle (*Cirsium arvense*), sow thistle (*Sonchus arvensis*), and dandelion (*Taraxacum* spp.). Dandelion crowns were usually rotted off by the pathogen, but several new shoots soon appeared from below ground. This apparently explains the ability of dandelions and other perennial weeds to recover quickly and take possession of killed out patches in stands of alfalfa, clovers, or grasses (3).

## Influence of Cultural Practices

### Crop Rotation and Pathogen Survival

Crop rotation is obviously a valuable method of controlling or reducing the damage caused by the winter crown rot pathogen. Spring sown cereals, flax, or other annual crops can be grown after a severely diseased stand is ploughed up, since only overwintering crops are attacked. Also, the foregoing results have shown that brome grass, crested wheat grass, or other highly resistant grasses can be safely planted in highly infested land. In order to determine how long it would be necessary to grow these resistant crops before the pathogen would disappear, studies were made on its survival under various cropping practices and conditions.

Uniformly infested land, obtained by killing a stand of alfalfa by artificial inoculation with the pathogen, was divided into replicated blocks in which alfalfa was replanted immediately and after intervals of one, two, and three seasons of summer fallow or a cereal crop. Oats and wheat gave similar results when used as the cereal crop in different experiments. The average survival in the replanted alfalfa was as follows.

| | |
|---|---|
| Alfalfa replanted in same season | None |
| After one season of summer fallow | 55% |
| After one season of cereal crops | 63% |
| After two seasons of summer fallow | 83% |
| After two seasons of cereal crops | 80% |
| After one season of summer fallow and one of cereal crops | 81% |
| After one season of summer fallow and two of cereal crops | 100% |
| After two seasons of cereal crops and one of summer fallow | 100% |

These results indicate that the decrease in viability of the pathogen is fairly rapid in infested land which is summer-fallowed or planted to a cereal crop. Relatively little damage occurred after two years of such treatment and none after three years. There were no consistent differences in survival following various combinations of summer fallow and cereal crop. Similar results were obtained when winter wheat was substituted for alfalfa in certain experiments.

Field observations also indicate that it is generally safe to replant alfalfa two or three years after a severely diseased stand has been ploughed up. The planting of resistant crops, however, does not always result in complete elimination of the pathogen. In one field under detailed study, a severely diseased stand of alfalfa was followed by a cereal rotation for five years before alfalfa was again planted. Winter crown rot was not observed in the new stand until the third season, when it began to develop slowly in the areas where it formerly occurred. Similar evidence obtained in other fields and plots where re-infestation seemed unlikely indicates that the pathogen may in some cases persist to a slight degree for several years in the absence of a susceptible host.

The decay of diseased alfalfa plants was studied in relation to survival of the pathogen. In repeated experiments, the base of the stems proved more infective than any other part of the diseased plant. As previously reported (3), this material is also most suitable for isolation of the pathogen. It is apparently harbored to a lesser extent in the diseased crown and upper portion of the root. When diseased plants were ploughed under in the spring, their remains were usually detectable in the soil for the balance of the season and occasionally in the following spring. They were seldom observed during the second season. In crock experiments, the decay of plants killed following inoculation was studied in soil maintained at different levels of moisture. After one year of storage at 17° C., the plant remains were not detectable, except in the air-dry soil, where they were preserved in a desiccated, shredded form. However, this dry material proved less infective than the moist and

wet soil from the other crocks when used as inoculum for alfalfa after storage for 18 months.

Viability tests were made at yearly intervals on natural inoculum consisting of soil and plant remains taken from damaged fields. Survival of healthy alfalfa plants inoculated with this material was as follows: inoculum stored one year—none; two years—32%; three years—100%. In isolation studies the pathogen was obtained with difficulty from this inoculum after storage for two years, and it did not grow after three years.

In studies on artificial inoculum, samples of the soil – corn meal inoculum used each fall in field experiments were stored in crocks at room temperature and under refrigeration, and were tested for viability at yearly intervals. The average survival in alfalfa inoculated with the material stored at room temperature was as follows: inoculum stored one year—none; two years—45%; three years—100%. Inoculum stored under refrigeration killed all plants for the first two years but caused no damage after three years of storage. Similar results were obtained when isolations were made from the stored inoculum.

## Forage Mixtures

Winter crown rot development was studied in several forage mixtures since observational evidence (3) indicated that less damage occurred in alfalfa grown in mixed stands with brome grass than when planted alone or with other grasses. Alfalfa, alsike clover, and red clover were seeded singly, and in various combinations with grasses, in replicated blocks of highly infested land. These mixtures contained the following grasses: brome grass, crested wheat grass, slender wheat grass, Kentucky blue grass, meadow fescue, creeping red fescue, timothy, and red top. In the following spring, the alfalfa and clovers were killed or suffered severe damage in all plots regardless of the mixture in which they were planted. For example, alfalfa was as severely damaged when grown with brome grass as with the highly susceptible red top. The brome grass, however, was not affected and it soon multiplied and filled in the areas formerly occupied by alfalfa. The other grasses and legumes included in the mixtures were also damaged to about the same degree as in the tests with pure stands (Table I). Similar results were obtained in other experiments where the distances of spread of killing was measured in blocks or rows of different forage mixtures. Brome grass and other resistant grasses reduced the spread of the pathogen only in mixtures already containing a relatively low proportion of plants of the susceptible species.

## Age and Condition of the Stand

The influence of the age of alfalfa on susceptibility was determined by inoculating plants of nine different varieties which had been planted in replicated rows for five successive years. In all varieties, the age of the stand did not have a significant effect on the degree of survival. The only consistent difference noted was a slightly higher percentage of survival in the older stands in which some of the high crowned plants had apparently escaped.

Observational studies indicated that alfalfa stands seeded late or with a companion crop tended to suffer more damage than when seeded early or without a companion crop. Any other conditions which weakened the plants also appeared to increase their susceptibility.

## Fertilizers

Commercial fertilizers were applied to naturally and artificially infested land prior to replanting alfalfa. Most of the experiments included different rates and combinations of the following materials: ammonium phosphate, ammonium sulphate, triple superphosphate, potassium sulphate, sulphur, and lime. None of these treatments caused a significant reduction in winter crown rot development, and sulphur actually increased the damage in some experiments. Further tests on a wide range of soil types are necessary, but the present results indicate that fertilizers do not have a beneficial effect, except to improve plant growth under certain conditions.

## Cutting and Mulching

The effect of cutting alfalfa at different times in the fall was studied by measuring the spread of killing in plots and rows. The average distance of spread during a five-year period was as follows:

| | |
|---|---|
| Tops uncut in fall | 6.9 in. |
| Tops cut in mid-August | 6.1 in. |
| Tops cut in mid-September | 5.6 in. |
| Tops cut in mid-October | 4.7 in. |

In most experiments, cutting of the tops in October significantly reduced the distance of disease spread, as compared to that in the uncut plots. The differences were less marked in the plots cut in August and September, in which there was sometimes an opportunity for regrowth.

Since the foregoing results indicated that uncut tops of alfalfa encouraged spread of the pathogen, attempts were made to determine whether a surface mulch would have the same effect. As previously shown (3), the pathogen does not spread as readily across bare soil as among plants in plots or rows. In two experiments, cut alfalfa tops were spread on the surface of the soil between a row of alfalfa and the inoculum, the latter having been placed in shallow trenches parallel to the row at distances ranging from one to five inches. The average survival in the mulched row, with inoculum at varying distances, compared to that in a row with bare soil between the inoculum and plants, was as follows:

| | | |
|---|---|---|
| 1 in. distance: | mulched—none; | bare— 10% |
| 2 in. distance: | mulched—14%; | bare— 29% |
| 3 in. distance: | mulched—65%; | bare— 86% |
| 4 in. distance: | mulched—76%; | bare—100% |
| 5 in. distance: | mulched—87%; | bare—100% |

These results indicate that the pathogen can spread to a greater distance and cause more damage to alfalfa when there is debris on the ground than when it is bare.

## Discussion

The results of the present study and previously reported observations on natural infection (3) indicate that the winter crown rot pathogen has a very wide host range. It has attacked cultivated and native species of many different genera and families of dicotyledons and monocotyledons. As a class, the legumes appear particularly susceptible since all species tested were damaged to varying degrees. The present study has dealt mainly with forage crops, but it is quite possible that this pathogen is also responsible for much of the so-called winter killing of horticultural plants in the central and northern areas of Western Canada. The wide distribution of the pathogen, even in virgin land (3), indicates that it occurs commonly in nature, but spreads rapidly and causes noticeable damage only under certain conditions, or when a highly susceptible host, such as alfalfa, is planted. The foregoing results indicate that various herbaceous native plants and weeds could be responsible for harboring the pathogen. Woody plants are apparently not susceptible.

The use of resistant varieties would be the best method of controlling winter crown rot damage in overwintering crops. In alfalfa, most of the commonly grown varieties are highly susceptible, but good progress is being made in a breeding program (1) in which *Medicago falcata* and derived varieties are being used as sources of resistance. Attempts are also being made to incorporate resistance to wilt and other important diseases in the hybrids. Siberian red clover is a valuable source of resistance, and resistant plants of alsike clover have been selected. Likewise, it is hoped that a satisfactory degree of resistance can be developed in red top, creeping red fescue, timothy, and other susceptible grasses.

Crop rotation is the most valuable cultural method of controlling winter crown rot. Spring sown cereals and other annual crops, or brome grass, crested wheat grass, and other resistant grasses can be safely grown in highly infested land. Such crops should also be grown on new breaking for at least two years (3). Fortunately, the pathogen does not survive to any extent for longer than from two to three years in the absence of a susceptible host. Thus, alfalfa or other susceptible crops can be replanted after a short rotation of cereals, summer fallow, or resistant grasses. Mixtures containing brome grass or other resistant grasses are also of value. These grasses will prolong the life of mixed pasture or hay stands in which alfalfa or clovers may soon be killed. Under some conditions, the presence of resistant grasses in a mixture also appears to retard the spread of infection in alfalfa or clovers (3).

Other cultural practices studied are apparently not of practical value in controlling winter crown rot. Fertilizers did not reduce the damage, although they may have an indirect beneficial effect in producing stronger plants under certain conditions. Late fall cutting of alfalfa tops retards the spread of the

pathogen, but it also predisposes the plants to winter injury, and cannot be recommended. Infection is also reduced when old tops and other debris are removed, and fields should be raked as clean as possible. Since infection is favored by a slowly melting snow cover (3), any measures which prevent an excessive accumulation of snow may reduce winter crown rot damage.

## Acknowledgment

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# AN INHIBITOR IN A NEW HOST OF TOBACCO RING SPOT VIRUS[1]

By M. Weintraub[2] and J. D. Gilpatrick[3]

## Abstract

Of four seedling-clones of *Dianthus barbatus* L. tested for susceptibility to tobacco ring spot virus, two were consistently infected, one occasionally missed infection, and the fourth was insusceptible. Symptoms of infection were primary local lesions, followed by systemic chlorosis and death of the growing point. Further adventitious growth was symptomless, but still contained the virus and was protected from reinfection. Intracellular inclusions of the amorphous type occurred in diseased leaves. Difficulty in transmitting tobacco ring spot virus from *D. barbatus* to tobacco was caused by an inhibitor in healthy *D. barbatus* leaves. Some of the physical characteristics of this inhibitor were studied. It had no effect on transmission of tobacco ring spot virus to *D. barbatus*, but in cucumber, *Datura stramonium*, and snapdragon, it caused either a delay of symptom expression or an insusceptibility of a proportion of inoculated plants. The inhibitor reduced infectivity of tobacco mosaic, cucumber mosaic, and tobacco etch viruses.

## Introduction

Previous attempts to infect *Dianthus barbatus* L. with the tobacco ring spot virus (10) have been unsuccessful. In the course of the present investigation, however, it was found that some seedlings of this species may be susceptible to the virus, and others are not.

## Materials and Methods

Seedlings of *D. barbatus* were propagated vegetatively as clones, identified as Db 15, Db 40, and Db 20. The tobacco ring spot virus used was kindly supplied by Dr. A. F. Ross, of Cornell University. The inoculum was prepared by grinding infective leaves with a pestle and mortar. Inoculations were effected by rubbing with a finger leaves lightly dusted with carborundum.

### Symptoms

*Tobacco* (*Nicotiana tabacum* var. Harrow Velvet)

Tobacco plants inoculated with this virus produced discrete local lesions in three to five days, followed by systemic symptoms of light green rings and line patterns. This acute phase, in which symptoms were obvious, was followed by the chronic, apparently recovered phase, in which no symptoms were ordinarily visible on the new growth.

*D. barbatus*

Of the four clones inoculated with tobacco ring spot virus, three proved to be susceptible. Db 15 developed primary symptoms on the rubbed leaves in

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three to four days, and Db 40 usually within seven days, while Db 20 required from 14 to 20 days, and occasionally did not become infected. Db 41 was insusceptible to the virus.

The syndrome in all the susceptible clones was very similar. First symptoms of infection were faint chlorotic spots and rings, about 0.5 mm. in diameter, on each rubbed leaf (Fig. 1). These spots gradually became more conspicuous and enlarged until some of them reached a diameter of 1 cm. Meanwhile, the tips of the inoculated leaves became necrotic. While the tip necrosis was advancing, all the leaves became twisted, the youngest most severely. The young leaves then wilted, and developed necrotic areas and streaks. Finally, the growing point died, while the tip necrosis on the rubbed leaves extended almost one-quarter down the leaf. The rubbed leaves persisted for several months, although the primary lesions spread along these leaves and became orange-yellow (Fig. 2).

Frequently the uninoculated lateral shoots of inoculated plants became systemically infected, and died at the growing point. For reasons not completely understood, this did not always occur, some lateral shoots escaping infection. It is believed that such factors as temperature and light were at least partially responsible for this somewhat erratic behavior, since more lateral shoots became infected during the late summer than in the subsequent winter months.

### Apparent Recovery and Protection in *D. barbatus*

Most of the inoculated plants with dead growing points soon began to grow again from adventitious buds, and produced a luxuriant growth within two to three months (Fig. 3). The leaves on these adventitious shoots appeared healthy, although a few could be distinguished from those on uninoculated healthy plants by a twisting of the younger leaves, and the slightly shorter mature leaves. Such apparent recovery is reminiscent of the course of the disease in tobacco plants (6, 7, 8, 10) since the recovered shoots were protected from reinfection by the tobacco ring spot virus, and the virus was recovered from the symptomless leaves, although in somewhat lower concentration.

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Fig. 1. Local lesions on inoculated leaves of *D. barbatus*, produced by tobacco ring spot virus, three days after inoculation.

Fig. 2. Same plant, in advanced stage of infection. Note dead growing point, and tip necrosis of inoculated leaves, 15 days after inoculation.

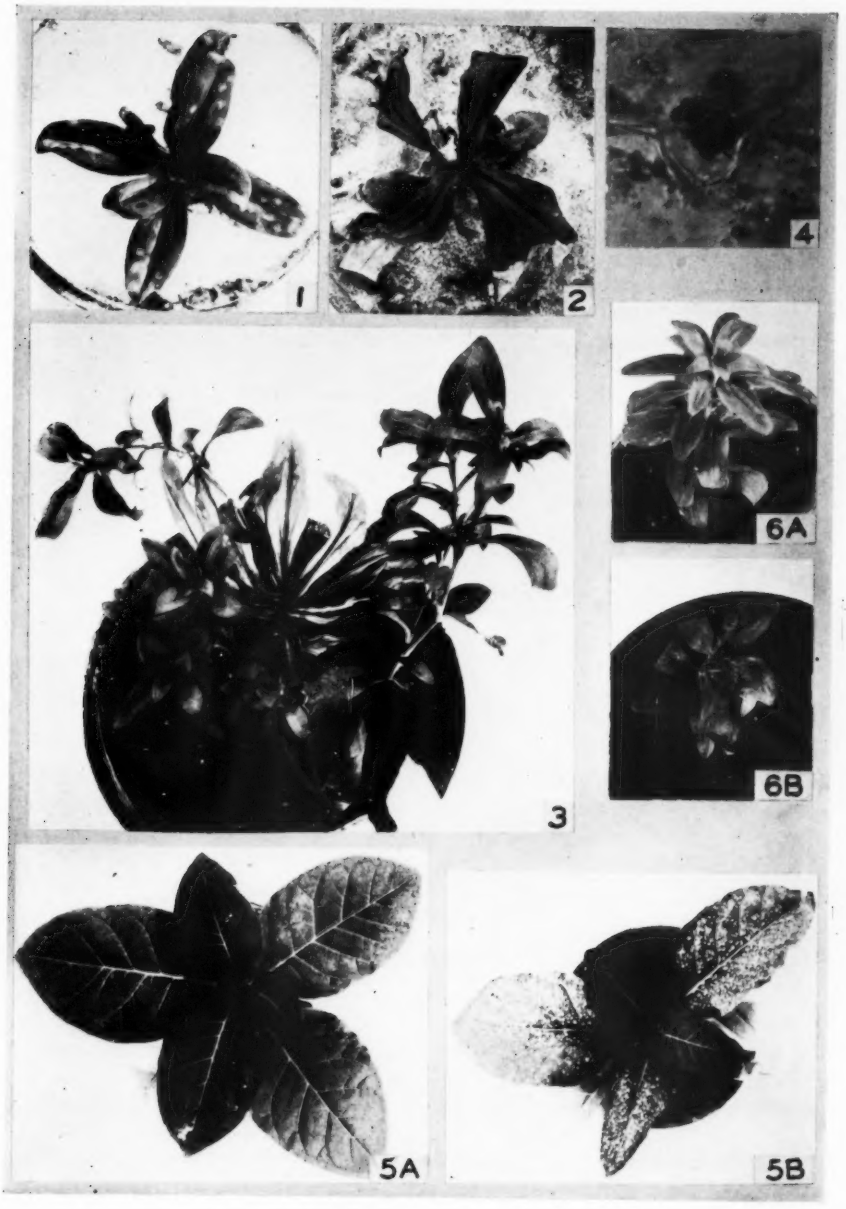
Fig. 3. *D. barbatus* recovered from tobacco ring spot disease. Note the apparently healthy adventitious shoots, and the persistent, yellowed leaves of the inoculated portion of the plant.

Fig. 4. Inclusion body, in epidermal cell of diseased *D. barbatus* leaf. The amorphous body is stained deeply, and is appressing the nucleus. ×1100.

Figs. 5A and 6A. Tobacco plant and snapdragon plant, respectively, inoculated with a mixture of tobacco ring spot virus and macerate of healthy leaves of *D. barbatus*. Note absence of infection.

Figs. 5B and 6B. Tobacco plant and snapdragon plant, respectively, inoculated with a mixture of tobacco ring spot virus and water. Note the numerous local lesions on the tobacco, and the circular lesions and dead growing point of the snapdragon.

PLATE I



### Intracellular Inclusions

Tobacco ring spot virus is known to induce the formation of intracellular inclusions in several of its hosts, including tobacco, *N. glutinosa*, and petunia, but rarely in cucumber (1, 11).

To determine whether such inclusions were produced by tobacco ring spot virus in *D. barbatus*, epidermal peelings of diseased *D. barbatus* leaves were stained by the method of McWhorter (5) and examined on the third day after inoculation. Amorphous bodies were present in abundance, usually appressing the nucleus, and easily distinguished by their deeper color (Fig. 4). For the most part, these bodies were irregularly circular in shape, 10 to 15 $\mu$ in diameter, but occasionally they were ellipsoidal, or kidney-shaped. Crystalline inclusions could not be found in any of the sections. Furthermore, examination of peelings from healthy plants showed that the amorphous bodies were not present.

When peelings were made of diseased leaves on the fifth and sixth day after inoculation, very few amorphous bodies could be found, and the outstanding cellular feature appeared to be a disorganization of the protoplast. Many cells were binucleate, and all contained great numbers of deeply staining granules, which were too small to be accurately resolved and described as crystals. Much of the granular debris was in loose masses, frequently grouped around the nucleus. This arrangement suggested that an amorphous body had ruptured and had given rise to the numerous granules. Comparable peelings of healthy leaves showed none of these phenomena, but only a protoplast that stained normally. Binucleate cells occurred occasionally in healthy tissue also, but their frequency was much lower than that in diseased leaves.

### Recovery of Tobacco Ring Spot Virus from *D. barbatus*

Attempts to transmit the tobacco ring spot virus from diseased *D. barbatus* to other hosts resulted in a gradation of successful transmissions, depending on the species of the test plant inoculated, and on the clonal source of the virus (Table I). Thus, tobacco is at one extreme of susceptibility (minimum), and *D. barbatus* at the other extreme of susceptibility (maximum), when *D. barbatus* is used as the source of the virus. The other three hosts appear to lie between these two extremes of susceptibility.

### Presence of an Inhibitor in *Dianthus*

These data suggested that an inhibitory substance, present in the leaves of *D. barbatus*, might be responsible for suppression or delay of virus multiplication. To test this possibility leaves of tobacco plants in the acute phase of tobacco ring spot disease were macerated and mixed with macerated leaves of healthy *D. barbatus*, Db 20. As a control, another portion of the diseased tobacco macerate was diluted with water. In this, and in all succeeding experiments, both the virus–inhibitor and virus–water mixtures were prepared in a 1:1 ratio. Each of these mixtures was rubbed on the leaves of eight tobacco, 10 cucumber, two snapdragon, and two *D. barbatus* plants.

TABLE I

TRANSMISSION OF TOBACCO RING SPOT VIRUS FROM THREE CLONES OF *D. barbatus* TO TOBACCO, *D. barbatus*, CUCUMBER, *Datura stramonium*, AND SNAPDRAGON

| Source of virus | Test plant | No. of plants infected | Delay of symptom expression beyond that of controls,* weeks |
|---|---|---|---|
| Db 20 | Tobacco | 2/8 | 2 to 7 |
| Db 40 | " | 5/11 | 3 to 7 |
| Db 15 | " | 7/11 | 2 to 4 |
| Db 20, 40, or 15 | Db 15 | 10/10 | — |
| | Db 40 | 17/17 | — |
| Db 20, 40, or 15 | Db 20 | 18/22 | — |
| Db 20 | Cucumber | 1/12 | 1 |
| | *Datura stramonium* | 4/4 | 2 |
| | Snapdragon | 4/4 | 1 |

* *Controls were the same species of plant inoculated with tobacco ring spot virus from tobacco.*

All of the plants inoculated with the virus–water mixture became infected. Of the eight tobacco plants inoculated with the virus–inhibitor mixture, six showed no symptoms, and two developed only a very few lesions (Fig. 5). Neither of the two snapdragon plants inoculated with the latter mixture showed any symptoms (Fig. 6), while only two of the 10 cucumber plants became infected, the symptoms developing two days after those on the virus–water controls. Both of the *D. barbatus* plants inoculated with the virus–inhibitor mixture became infected.

It was considered of interest to determine whether this inhibitor was restricted to the *barbatus* species, or whether it was common in the genus *Dianthus*. Accordingly, macerates of diseased tobacco leaves were mixed with macerates of healthy leaves of *Dianthus caryophyllus*, *D. chinensis*, *D. heuteri*, *D. caesius*, *D. barbatus*, Db 15, and Db 40. Each of the mixtures was rubbed on the leaves of two healthy tobacco plants. At the same time two tobacco plants were inoculated with a virus–water control mixture.

None of the virus–healthy leaf mixtures produced infection, with the exception of the virus – Db 40 mixture. The two plants inoculated with the latter mixture developed a mild systemic infection after six weeks. The plants inoculated with the virus–water mixture both developed numerous local lesions and severe systemic symptoms.

Thus, it appeared that the inhibitor was generally present in the leaves of members of the genus *Dianthus*. The results with the three clones of *D. barbatus* would indicate, however, that the inhibitor may not be present in the same concentration in all seedling clones.

## Properties of the Inhibitor

Experiments were set up to determine some of the physical characteristics of the inhibitor present in *D. barbatus*. Appropriate controls were run with each test, so that virus–inhibitor mixtures were always compared with virus–water mixtures of the same dilution. Tests were repeated at least once, and some, twice. In the following sections, the term "virus-macerate" refers to the macerate of tobacco leaves in the acute phase of tobacco ring spot, and "inhibitor-macerate" to the macerate of healthy *D. barbatus*, Db 20, leaves.

### *Dilution End Point*

Inhibitor-macerate was diluted to $10^{-1}$, $10^{-2}$,and $10^{-3}$ with distilled water. Each of the preparations was mixed with virus-macerate, and used to inoculate two tobacco plants. The (virus–inhibitor $10^{-1}$) mixture infected one out of two plants. Symptoms were not manifest until five weeks after inoculation and consisted of two doubtful local lesions and mild systemic infection. The (virus–inhibitor $10^{-2}$) mixture produced a number of local lesions in both plants three days after inoculation, but fewer than in the control plants. This was followed by a relatively severe systemic infection. The (virus–inhibitor $10^{-3}$) mixture reacted exactly as did the control mixture, producing numerous local lesions three days after inoculation, which were followed by severe systemic symptoms. The dilution end point of the inhibitor was, therefore, between $10^{-2}$ and $10^{-3}$, although it should be noted that even at $10^{-1}$ inhibition was incomplete. Thus, the severity of infection, and the length of time before appearance of symptoms was directly proportional to dilution of the inhibitor.

### *Resistance to Aging in vitro*

The inhibitor-macerate was allowed to stand in a test tube at room temperature, and mixed with virus-macerate at 0, 24, 48, and 72 hr. Each mixture was tested on three tobacco plants. Since tobacco ring spot virus is relatively unstable *in vitro*, a fresh virus preparation was made for each of the four mixtures, an appropriate virus–water mixture being tested at the same time.

It was found that the inhibitor began to lose its effectiveness after 24 hr., so that, 48 hr. after preparation of the inhibitor-macerate, all three tobacco plants became systemically infected 12 days after inoculation. After 72 hr., the inhibitor had no effect.

### *Thermal Inactivation Point*

Portions of inhibitor-macerate were heated for 15 min. at 60, 70, 80, 90, and 100° C. Each portion was then mixed with virus-macerate. The inhibitory effect was absent at, and above, 80° C., while at 60 and 70° C. there were produced no primary lesions and only mild systemic symptoms, delayed a few days beyond appearance of systemic symptoms in the control plants.

### *Resistance to Drying in vitro*

A portion of inhibitor-macerate was oven dried at 45° C., for 90 min. The dried residue was taken up in sufficient distilled water to make up the original volume. This macerate was then mixed with virus-macerate and tested for

inhibition. It was found that drying in no way impaired the effectiveness of the inhibitor, since neither local lesions nor systemic symptoms appeared in the inoculated plants.

*Effect of Centrifugation*

The effect of centrifugation on the inhibitor was tested in two ways, both of which yielded identical results. In the first method, a portion of inhibitor-macerate was centrifuged at 12,000 r.p.m. for 30 min. The resulting supernatant and precipitate were each mixed with virus–macerate and tested for inhibition on tobacco and snapdragon. In the second method, virus-macerate was mixed with inhibitor-macerate, and then centrifuged at the same speed as before. The precipitate was suspended in a minimum of water, and supernatant and precipitate portions were each tested for inhibition.

With both methods, the virus–precipitate mixture showed inhibition to the same extent as the control mixture of virus–uncentrifuged inhibitor, on both tobacco and snapdragon. The virus–supernatant mixture apparently contained considerably less inhibitor, since on tobacco plants it induced a few local lesions, which were followed by systemic symptoms 10 days after inoculation. The snapdragons, however, became systemically infected, at the same time and as severely as the controls.

*Effect of Dialysis*

In this experiment, also, both the inhibitor-macerate alone, and a mixture of virus–inhibitor were used. The preparations were dialyzed against running tapwater for six hours. In neither case did the inhibitor lose its effectiveness. To ensure that dialysis for six hours did not inactivate the virus itself in the virus–inhibitor mixture, an additional control was run in which a portion of virus–water mixture was dialyzed simultaneously. This treatment had no effect on the infectivity of the virus.

*Stability at Various pH Levels*

Leaves of healthy *D. barbatus* were ground in phosphate–citrate buffer at pH 3, 4, 5, 6, 7, and 8, and in phosphates–odium hydroxide buffer at pH 11, 11.3, and 11.8. The macerates were allowed to stand for 30 min., after which the pH of each was tested. It was found that at the end of this time, the pH of the macerates did not differ perceptibly from the pH of the corresponding buffer alone. Each of the macerates was then adjusted to pH 7, mixed with virus-macerate, and tested for inhibition. Of the nine mixtures, only that at pH 3 had lost its inhibitory activity, the other eight producing no symptoms whatsoever.

*Effect of Separate Application of Inhibitor and Virus*

It was not, as yet, known whether the inhibitor acted directly upon the virus, or upon the host, beyond the fact that a mixture of virus and inhibitor *in vitro* resulted in almost immediate inhibition of infectivity. To test the effect of applying the inhibitor and virus separately to the host plant, four tobacco plants were rubbed with inhibitor-macerate, rinsed thoroughly with water,

and immediately inoculated with virus. A similar set was rubbed with inhibitor-macerate, and inoculated two hours later. A third set was inoculated with virus, and rubbed with inhibitor-macerate two hours later. The results of all three tests were identical in the total absence of inhibition.

*Effect of the Inhibitor on Other Viruses*

Inhibitor–macerate from Db 20 was tested with three other viruses: tobacco mosaic virus, partially purified; cucumber mosaic virus, isolated from gladiolus (2); and tobacco etch virus, kindly supplied by the Dominion Laboratory of Plant Pathology, Harrow, Ont.

Tobacco mosaic virus was tested by the half-leaf method on five *N. glutinosa* plants, an equal number of left and right halves of 15 leaves being inoculated with the virus–inhibitor mixture and the control virus–water mixture. Local lesions were counted on the fifth day after inoculation. The effect of the inhibitor was not absolute, since some lesions were produced by the virus–inhibitor mixture. However, the difference between corresponding halves was highly significant, indicating that the virus had been inhibited to a considerable extent.*

The other two viruses, cucumber mosaic and tobacco etch, were completely inhibited when mixed with inhibitor–macerate, since the four tobacco plants inoculated with virus–inhibitor mixtures remained healthy, while the control plants, inoculated with virus–water mixtures, all became infected.

## Discussion

It is evident from the data presented that some clones of *Dianthus barbatus* can be hosts for tobacco ring spot virus, while others are apparently immune. Wingard's failure to infect this species (10) could, therefore, be explained by varietal differences in susceptibility.

The presence of the inhibitor in *D. barbatus* may, to some extent, account for differences in susceptibility of seedlings. It has been shown that the macerate of healthy leaves of Db 20 was more effective in inhibiting tobacco ring spot virus than the macerate of Db 40. Furthermore, it was more difficult to transmit the virus from Db 20 than from Db 15 or Db 40 to tobacco. This suggests that the inhibitor is present at a lower concentration in Db 15 and Db 40 than in Db 20. Such a suggestion is, of course, purely speculative, since there is no direct evidence to indicate that virus and inhibitor come in contact *in situ*.

No definite conclusion could be reached as to whether the inhibitor acted directly on the virus, or on the host. The only tests that could be interpreted in favor of the former were those in which separate application of inhibitor and virus resulted in complete absence of inhibition. This may be taken to indicate that the virus must come into contact with the inhibitor before inhibition can occur. It is interesting to note that these results are in

* *Mean number of lesions on the virus–water halves* $= 92.5 \pm 2.93$.
*Mean number of lesions on the virus–inhibitor halves* $= 9.75 \pm 0.54$.
*Difference of means* $= 82.75 \pm 2.96; P < .001$.

direct contrast to those obtained by Gupta and Price (4), who were able to inhibit tobacco mosaic virus and southern bean mosaic virus by spraying a filtrate of *Tricothecium roseum* on the lower surface of bean leaves, followed by inoculation of the upper surface with the virus.

In attempting to formulate the mechanics of inhibition by the *D. barbatus* inhibitor, it is necessary to keep in mind the different reactions of the various host species, with tobacco at one extreme of sensitivity (maximum), and *D. barbatus* at the other extreme of sensitivity (minimum), to inhibition. This phenomenon has been reported by previous investigators, including Bhargava (3) and van der Want (9). Bhargava observed that it was more difficult to infect tobacco with cucumber mosaic virus from sugar beet and *Phytolacca*, both of which contain inhibitory substances, than it was to infect the inhibitor-containing hosts themselves. van der Want reported that the sap of healthy carnation leaves was able to inhibit tobacco necrosis virus, tobacco mosaic virus, and rattle virus. This inhibitor is probably identical with the one described in the present paper. van der Want suggested that this inhibitor may be responsible for difficulty in transmitting carnation mosaic virus from carnation to aster, tobacco, and French bean, but, since there is no effect when the same virus is transmitted from carnation to carnation or to sweet William, he called this inhibitor a "relative" one, to distinguish it from "absolute" inhibitors, such as tannins.

If the hypothesis that the inhibitor affects the host is true, it is necessary to visualize a differential reaction on different host species, and, in the case of *D. barbatus*, on different clones of the same species. Such a mechanism could affect host susceptibility to make tobacco almost insusceptible to infection, and leave clones Db 15 and Db 40 of *D. barbatus* entirely unaffected.

The alternative hypothesis, that the inhibitor acts directly on the virus, suggests several possible mechanisms. One such is that the inhibitor enters into a loose inactivating complex to the same degree with any of the different viruses. The various host species are then capable of breaking this complex, and reversing the inactivation, to a greater or lesser degree according to the species of plant. That the virus and inhibitor are actually in a loose reversible relation is evidenced by the fact that centrifugation of the virus–inhibitor mixture succeeded in ridding the supernatant of inhibitor, leaving it infectious.

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# PHYSIOLOGICAL AND BIOCHEMICAL STUDIES IN PLANT METABOLISM

## VI. THE EFFECT OF ONTOGENY ON THE PHYSIOLOGICAL HETEROGENEITY IN THE FIRST LEAF OF WHEAT[1]

By D. W. A. Roberts[2]


### Abstract

The respiration rate, R.Q., and the water content of each of the developing quarters of the first leaf of Khapli wheat have been measured at different ages and found to change as the tissue ages. In general the respiration rate declines as the tissue ages. The R.Q. is usually close to unity except in the basal quarter around the fifth day. The water content of the quarters tends to decline as the mature leaves become older. In the basal quarter of four- and five-day-old leaves the oxygen uptake appears to reach its peak when calculated on a dry weight basis. In the five-day-old leaves the rate of water uptake is very high and the R.Q. very low. These properties are thought to be correlated with the phase of elongation which is occurring in this quarter at that stage.


### Introduction

In earlier papers of the present series (3, 4) the effects of illumination and soil water on the physiological heterogeneity of the first leaf of wheat have been considered. In the present paper the changes which accompany growth will be considered. Ontogenetic changes in the respiratory gradient during the early period of growth are to be expected since it is well known that very high and rapidly changing respiration rates occur in plant tissues containing actively dividing and maturing cells.

The plants were grown in soil under standardized artificial conditions as described earlier (1, 2). The leaves were harvested after two hours of darkness. Water content, respiration rate at 22.2° C. measured in terms of oxygen uptake, and R.Q. were determined by the methods described in an earlier publication (2). The respiration rates are average values for the first three hours of starvation after harvesting instead of the "stabilized rates" (3) used in the earlier papers of this series.

### Changes in Morphology

The development of the leaves has been described previously by Duff and Forward (1). In the present connection it is necessary to consider only the tissues used for the respiration samples where they include more than just the first leaf tissue and also the particular quarters in which mitosis and elongation are occurring. The first leaf of wheat is produced by a basal meristem so that the youngest tissue is found at the base of the leaf. In all cases the first leaf

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tissue was separated from coleoptile tissue. However in the three- and four-day-old leaves the apical meristem and the second leaf were not separated from the first leaf tissue. The respiration of these tissues is therefore added to the basal quarter of the first leaf tissue. No separation of these tissues was made owing to the small size and delicacy of the parts involved. In all leaves older than four days the tissue considered is only that from the first leaf. By using the Feulgen stain technique it was found that cell division is taking place only in the basal quarter of four-day-old leaves and not at all in older leaves. In the four-day-old leaves the continuity of the xylem elements through the base of the leaf is clearly visible.

The general procedure in carrying out the respiration experiments was to make the determinations of gas exchange one day, and to clean out the flasks on the following day. When the quarters were removed from the flasks, it was noted that the fourth quarters looked longer than the other quarters. In order to check this, all quarters of four-, five-, and six-day-old leaves were measured and weighed on removal from the flask. The results, given in Table I, show that the basal quarter of four- and five-day-old leaves is much longer than the two upper quarters. Since quarters one and two are nearly

TABLE I

THE AVERAGE LENGTHS OF WHEAT LEAF QUARTERS AFTER 24 HR. IN DISTILLED WATER AT 22.2°C.

| Age in days | Number of leaves | Average length of quarter in cm. | | | |
|---|---|---|---|---|---|
| | | I | II | III | IV |
| 4 | 17 | 1.08 | 1.12 | 1.16 | 1.51 |
| | 18 | 1.56 | 1.53 | 1.61 | 1.95 |
| | 29 | 0.92 | 0.98 | 1.08 | 1.46 |
| 5 | 18 | 2.05 | 2.03 | 1.99 | 2.38 |
| | 22 | 2.29 | 2.31 | 2.27 | 2.49 |
| | 19 | 2.46 | 2.52 | 2.47 | 2.87 |
| 6 | 18 | 2.89 | 2.92 | 2.89 | 3.01 |
| | 16 | 3.27 | 3.29 | 3.34 | 3.50 |
| | 18 | 2.92 | 2.96 | 2.94 | 2.99 |

equal in length it is concluded that they give an index of the initial length of all quarters. Consequently it is evident that the basal quarter of four- and five-day-old leaves has undergone a pronounced elongation. Table II shows that a marked water uptake occurs in the same quarters as the pronounced elongation. Similar events are taking place on a much smaller scale in the third quarter of four-day-old leaves, and a little elongation is occurring in the basal quarter of six-day-old leaves. In general, it will be noted that parts of these young leaves tend to take up large quantities of water, as would be expected of growing tissues taken from the region of elongation.

TABLE II

THE UPTAKE OF WATER BY WHEAT LEAF QUARTERS IN THE FIRST 24 HR. IN DISTILLED WATER AT 22.2° C.

| Age in days | Number of leaves | Water uptake for quarters, % initial fresh weight | | | |
|---|---|---|---|---|---|
| | | I | II | III | IV |
| 4 | 18 | 4.7 | 11.8 | 18.6 | 52.5 |
| | 29 | −5.7 | 2.2 | 11.4 | 50.7 |
| 5 | 22 | −0.7 | 1.0 | 6.6 | 29.0 |
| | 19 | 9.6 | 10.3 | 10.2 | 15.0 |
| 6 | 16 | 3.6 | 4.0 | 3.3 | −7.8 |

## Changes in the Rate of Oxygen Uptake and R.Q.

Fig. 1 shows the ontogenetic changes in the average value of the respiration rate for the first three hours of starvation after quartering. The respiration rate drift of the quarters with age is broadly similar to that of whole leaves, inasmuch as all exhibit a rapid decline during growth from early high values to much lower values later. Between days 9 and 12 a slight increase in the respiration rate of all quarters is shown. These data confirm the conclusion of Duff and Forward (1) that there is a transitory rise in the respiration rate of whole leaves at this time. In the early stages of ontogeny, the respiration rates of the basal quarters are much greater than those of the apical tissues, and the rapid decline in the respiration rate of the basal quarters occurs later in time than does that in the terminal region. This undoubtedly is a result of the leaves possessing a basal meristem which creates a gradient in age in the cell population, the youngest with the highest rates of respiration being in the basal quarter and the oldest with the lowest rates in the terminal quarter.

In two places the drift of oxygen uptake in the quarters with time differs from that of whole leaves. In the fourth quarter, the rate of oxygen uptake is higher on the fourth day than on the third day. In the tip quarter, the rate of oxygen uptake with time rises slowly and steadily with increasing age after the 13th or 14th day. The possible significance of these two points will be discussed later.

In Fig. 2 the average rates of oxygen uptake by the leaves are plotted so that the respiratory gradients are revealed. In these graphs it should be noted that the scale used for plotting the gradients in the three-, four-, and five-day-old leaves is five times that used for the seven-day-old leaves and all older leaves, whereas the scale for the six-day-old leaves is two times that used for the older leaves. This tends to obscure the great contrast between the gradients in the young leaves and those in the mature leaves. In the young leaves, the gradients are very much steeper than in the older leaves. Leaves in the three-, four-, and five-day-old juvenile stages are characterized by high rates of oxygen uptake, steep respiratory gradients in which the greatest rate usually occurs in the basal quarter, and the lowest usually in the tip quarter.

The gradients at this stage are probably due to the distribution of cells in the leaf which are undergoing division, vacuolation, and maturation.

After the juvenile stages, characterized by their high respiration rates and steep gradients in rates of oxygen uptake, have passed, the gradient passes through a somewhat variable period which persists until after the early stages

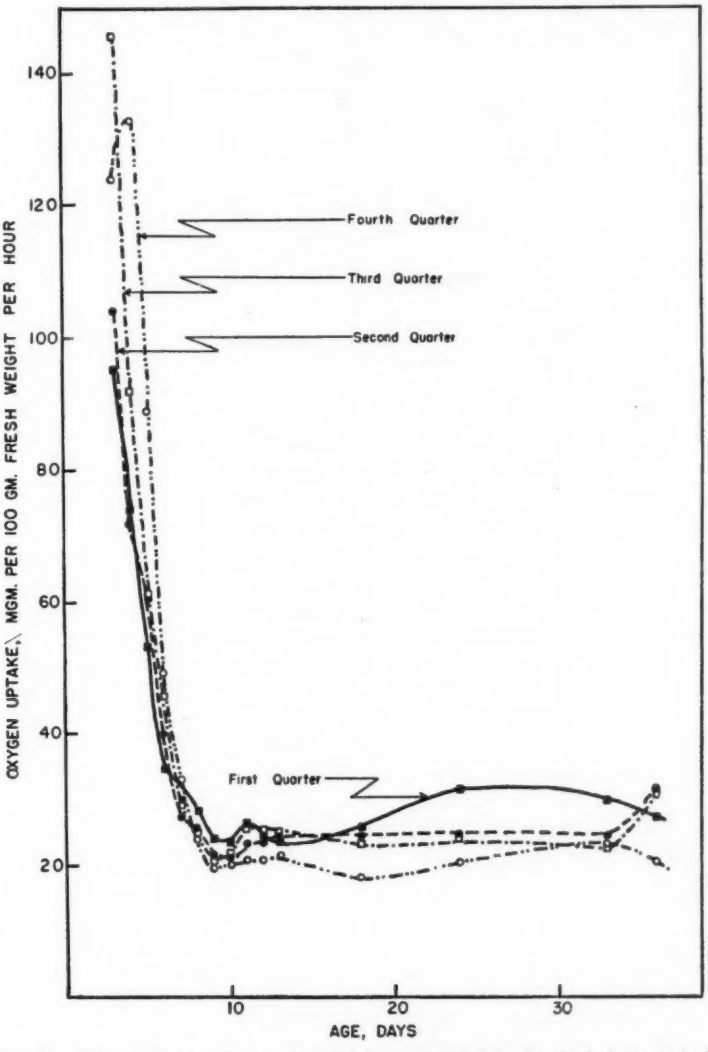


FIG. 1. Changes in the oxygen uptake of the quarters of the first leaf of wheat during ontogeny.

of maturity. During this period it will be seen from Fig. 1 that the respiration rates of the different quarters lie close together and their graphs intersect each other at various points. There is no reason for supposing that small differences

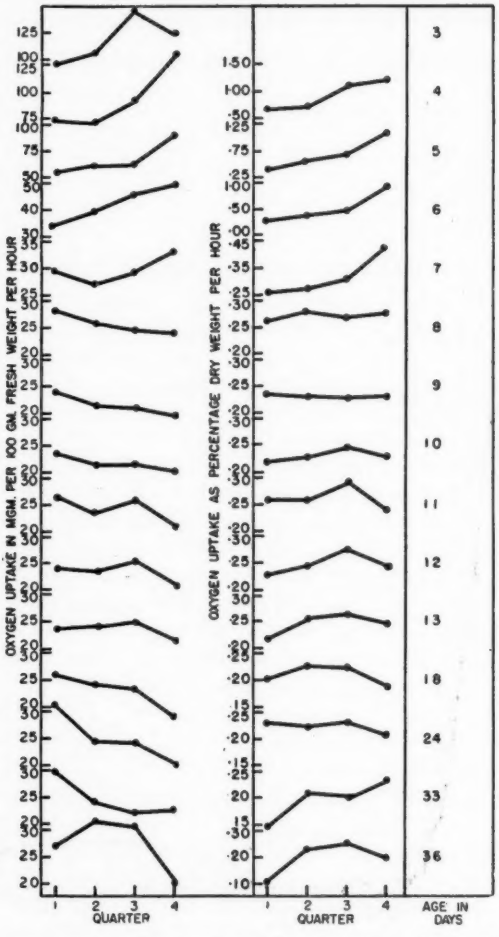


FIG. 2. The gradients in oxygen uptake of the first leaf of wheat during ontogeny.

in the respiration rate of a given quarter at a given age do not occur during this period and that some variations in the gradient and timing of the changes in the gradient will not occur during this period. In considering the following discussion of these stages in ontogeny this variability should be borne in mind.

The six- and seven-day-old leaves are regarded as the beginning of the transitional stage. During these two days the gradient becomes much less

steep and gradually takes on the form which appears in the 8-, 9-, and 10-day-old leaves. At this stage there is a uniform gradient in the leaves from tip to base, with the greatest respiration rate in the tip of the leaves, and the least in the base.

Under the particular conditions of my experiments the leaves pass into the mature condition in which the rate of oxygen uptake is least in the basal quarter, most in the third quarter, and only slightly less in the second and tip quarters. As the leaves pass into the late stages of maturity, a tip to base gradient similar to that described for the 8-, 9-, and 10-day-old leaves appears, and gradually becomes stronger. Finally the respiration rate in the tip quarter falls owing to the withering which occurs there in old age. This phenomenon is not apparent until about the 36th day is reached. It will be noted that the over-all effects of ontogeny result in a complete reversal of the respiratory gradient (cf. 6-day stage and 24-day stage) and a very great reduction in its slope between the juvenile stages and maturity.

In the above account the changes in the gradient following day 11 have been described as if they were attributable solely to the aging of the leaves. There is, however, a second possibility. When the sequence of changes in the gradient is compared with that of the gradients produced by increasing soil water reported earlier (4), a striking similarity is noted. During the growing of the plants, a constant amount (60 ml.) of water was added to each can every day. In those cases where the plants were not harvested before three weeks, it was apparent that the soil was becoming steadily wetter.

It is therefore quite possible that the changes in the gradient following day 11 are due to changes in the soil water rather than to age. This may account for the increasing respiration rate in the tip quarter after day 13 or 14. In the discussion of the effect of ontogeny upon wheat leaf respiration Duff and Forward (1) reported that the respiration rate dropped slowly after about day 12 or 13. In the present series of experiments this drop in the respiration rate was not found. However the procedure of growing the plants for these experiments differed from that of Duff and Forward in that the latter authors varied the water supply to the cans to keep them reasonably moist, instead of adding a stated quantity of water each day as has been done in the present set of experiments. This may account for the difference between the two sets of data.

The general picture presented by the respiration rates calculated on the basis of dry weight is similar to that given for the rates calculated on the basis of fresh weight. The most notable discrepancy between these two pictures is the small drop in the respiration rate in the fourth quarter between day four and day five as compared with the much greater drop when calculated on the fresh weight basis. This is probably associated with the same phenomenon which causes the increase in rate of oxygen uptake in the fourth quarter between days three and four. This matter will be considered later.

Fig. 2 shows the respiratory gradients in the wheat leaf for the dry weight respiration rates. Once again in the young leaves, the steep gradient in

oxygen uptake from tip to base with the maximum values at the base of the leaves appears. This type of gradient persists until the seventh day, after which it passes gradually into the mature type of gradient with low rates in the basal and tip quarters, and the highest rates in the third quarter. Finally in old age the rate for the upper quarter drops to quite low values as that quarter withers and dies.

When the sequence of changes in the gradient after day 11 described above is compared with that of the corresponding series for plants supplied with varying amounts of soil water, a general similarity is seen and the agreement is nearly as good as in the case of the respiration rates calculated on the basis of

TABLE III

THE RESPIRATORY QUOTIENTS OF THE QUARTERS OF THE FIRST LEAF OF WHEAT AT DIFFERENT STAGES OF ONTOGENY

| Age in days | Value of respiratory quotient for quarter | | | |
|---|---|---|---|---|
| | I | II | III | IV |
| 3 | .95 | .98 | 1.06 | 1.09 |
| | .91 | .89 | .93 | — |
| | .97 | 1.00 | 1.00 | .95 |
| 4 | .99 | 1.01 | .96 | .93 |
| | 1.05 | 1.02 | .94 | .95 |
| | 1.20 | 1.08 | .95 | 1.06 |
| 5 | 1.05 | 1.00 | .93 | .76 |
| | 1.02 | 1.00 | .95 | .87 |
| | .95 | — | .93 | .83 |
| 6 | 1.02 | 1.05 | .98 | .93 |
| | .96 | 1.01 | 1.02 | .99 |
| | 1.13 | .99 | .96 | .89 |
| 7 | .95 | 1.06 | .98 | .86 |
| | .92 | .93 | 1.00 | 1.00 |
| | 1.04 | 1.06 | .99 | .89 |
| 8 | .98 | 1.02 | 1.06 | 1.01 |
| | — | .99 | 1.00 | .98 |
| | .97 | 1.01 | 1.05 | .97 |
| 9 | .96 | .98 | 1.00 | 1.04 |
| | .92 | 1.03 | 1.04 | 1.06 |
| | .93 | 1.02 | 1.09 | 1.05 |
| 10 | .94 | 1.03 | 1.02 | 1.05 |
| | 1.09 | 1.15 | 1.24 | 1.29 |
| | .93 | .95 | .98 | .98 |
| 11 | .97 | 1.05 | 1.07 | .94 |
| | .99 | 1.00 | 1.02 | 1.10 |
| | .99 | 1.02 | 1.00 | 1.06 |
| 12 | .99 | 1.02 | 1.07 | 1.05 |
| | .95 | — | .98 | .99 |
| | .96 | 1.03 | 1.04 | 1.03 |
| 13 | .97 | 1.00 | .99 | .98 |
| | .98 | 1.10 | 1.03 | .98 |
| | 1.03 | 1.06 | 1.10 | 1.08 |
| 36 | 1.03 | 1.10 | 1.10 | 1.15 |

fresh weight. It was found that the gradients for 24-day-old leaves are very similar to those of plants supplied with 75 ml. of water per day.

An examination of the data in Table III shows that the R.Q. of the different quarters of the wheat leaf rarely departs greatly from unity. In mature leaves the R.Q. of the tip or first quarter is usually slightly below unity, while the values for the other quarters are usually somewhat greater than unity. This difference in the R.Q. of the quarters can only be explained on the basis of differences in the course of metabolism in the different quarters. Since very little is known about the enzymic machinery of these leaves, no effort was made to try to determine the specific causes of the very small differences in R.Q. observed in the different quarters.

A further consideration of the data reveals that the R.Q. values for the different quarters change with age in a way characteristic of the particular quarter being considered. The value for the tip quarter is consistently below unity, except for a short period between four and six days after the planting of the seed. The R.Q. of the second quarter changes very little with age and lies consistently above unity. The R.Q. of the third segment of the leaf is above unity except for a period between the fourth and seventh day. In the fourth quarter the R.Q. at the three and four day stage is about unity and drops to quite low values by the time day five is reached. A possible correlative of this will be considered later. Following the fifth day the R.Q. of the basal quarter rises until the ninth day when it slightly exceeds unity. The subsequent values of the R.Q. of the ligule quarter are somewhat erratic but in general they exceed unity.

Thus the cell population of the leaf manifests metabolic heterogeneity in the respiratory quotients of its quarters. The slight but appreciable gradient of the R.Q. changes its sense twice in the observed course of ontogeny, appearing to pivot about the steady values of the second segment. The period of development lying between these two shifts, during which the value of the R.Q. lies a little above unity in the tip quarter and well below unity in the ligule quarter, corresponds to the latter part of the period of growth following the emergence of the leaf from the coleoptile. At this stage the elongating phase must be regarded as the dominant phase of growth though probably not the only growth activity in progress. No metabolic interpretation of this pattern of ontogenetic change can yet be offered. It is undoubtedly the outward indication of the changing course of metabolism that accompanies the development of the leaves and is therefore almost certainly determined by factors originating in ontogeny.

Fig. 3 shows the first 10 hr. of the air lines wheat leaf quarters at various stages of development. These graphs show that the slope of the air lines gradually becomes less steep as the leaves age. The transposition (cf. 2) is also seen in all quarters at the eight day stage and in the tip and second quarter at the seven day stage. The transpositional hump (cf. 2) appears in the two tip quarters at the 10 or 11 day stage. The respiratory record of the 7-day-old leaves shows quite clearly the age gradient among the cells of the

leaf. The air line of the tip quarter is quite similar to that of the mature leaves while the air line of the basal quarter is like that of the younger leaves. The sudden rapid increase in the oxygen uptake of four- and five-day-old leaf basal

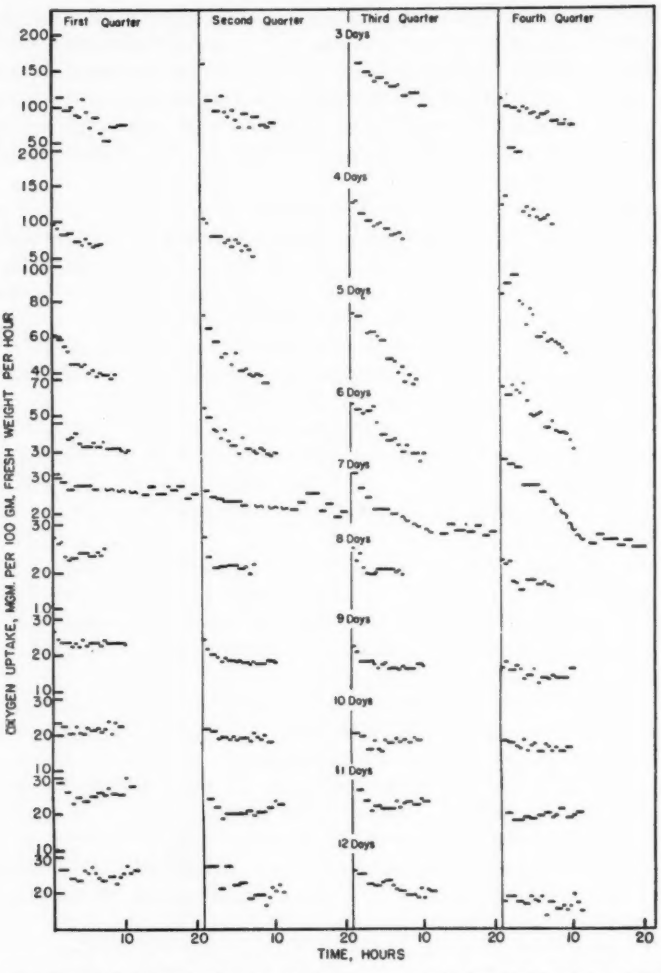


FIG. 3. The drift of respiration rate of the quarters of the first leaf of wheat of different ages during starvation.

quarters has not been clearly observed in parallel experiments on the air lines of these quarters at this stage and the available data suggest that it is an unusual occurrence.

A set of experiments similar to the foregoing has been made on the R.Q. records of the leaves at various ages. Regardless of the initial value of the R.Q. it was found that the respiratory quotient of all the quarters declined slowly with time, after the harvesting of the sample. In all quarters older than nine days the picture obtained was almost identical with that described in an earlier paper dealing with whole leaves (2).

## Changes in the Water Content

Fig. 4 is a plot of the average values of the water contents of the different quarters as they grow older. After about day 10 or 11 the water content of the leaves falls as the leaves age. This drop in water content is most marked in the tip quarter and least marked in the basal quarter. This is similar to the effect of increased soil water on the water content (4), and the increasing water content of the soil may be partly responsible for it. During the juvenile stages of ontogeny, a rather complex series of changes is seen to occur. The most striking of these changes occurs in the basal quarter, where the water content reaches 95% at the six-day-old stage.

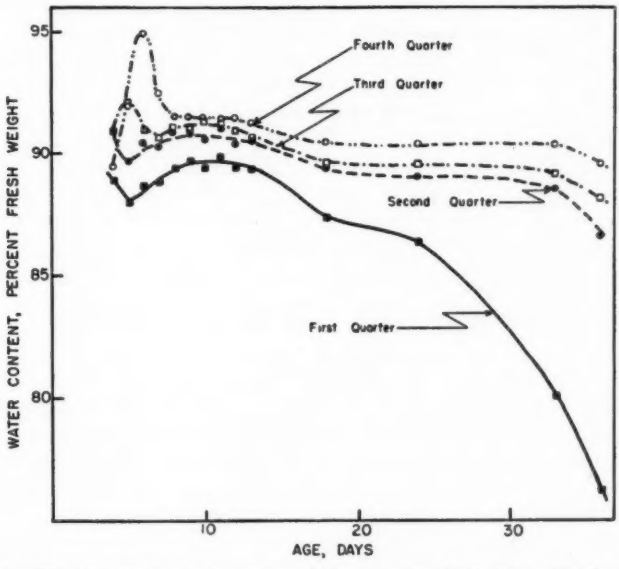


FIG. 4. The changes in water content of the quarters of the first wheat leaf during development.

Fig. 5 shows the water content gradient of the leaves at different ages. Except in the four-day-old leaves this gradient does not change in direction and the tip quarters always contain the least water and the basal quarters the most water. This state of affairs would be expected since the basal quarter

is nearer to the source of supply of water. The gradient is steep at the six day stage just after the basal quarter has finished elongation. In old age the water content gradient becomes steep again owing to the withering of the tip quarter and its consequent loss of water.

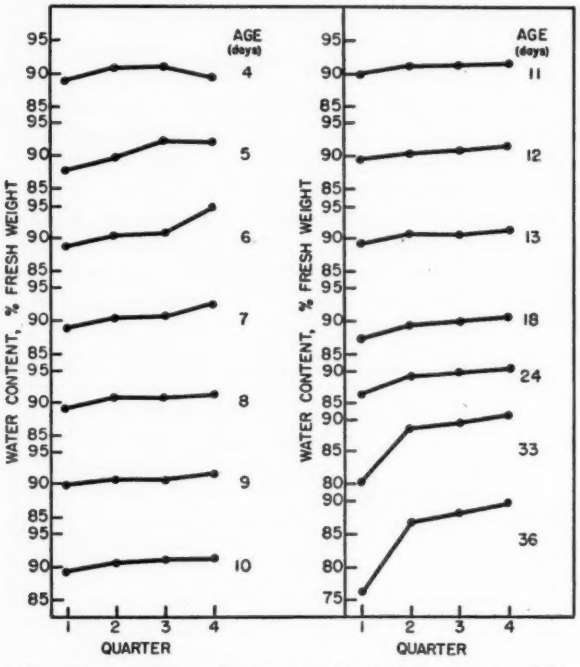


FIG. 5. The water content gradient in the first leaf of wheat at different ages.

## Discussion

Attention has been drawn to the peculiar behavior of five-day-old basal quarters of the leaves, in several connections. These quarters have a high rate of oxygen uptake if calculated on the basis of dry weight when they are five days old. They also possess an unusually low R.Q. lying between 0.75 and 0.9. Direct observation shows that little active cell division is occurring in the basal quarters at this stage. However, it has been shown (Tables I and II) that water uptake and elongation are still actively in progress at this stage. The very great increase in water content in the basal quarter between days four and six shows that water uptake must be quite actively going on in the attached five-day-old basal quarters. Since all these events coincide in time, it is suggested that the process of elongation in the leaves is probably accompanied by very high rates of oxygen uptake and low values for the R.Q. Whether this peculiar type of metabolism is due to the stretching of the cell

walls, vacuolation, mineral uptake, or water uptake, or a combination of some or all of the above activities can not be decided from the experimental data on hand. If these peculiarities are due to cell elongation, one would expect to find them in the other quarters. There is a distinct indication of these events in the third quarter at the four or five day stage. In the other two quarters, these events would occur at a still earlier time, and in the tip quarter the external appearances indicate that elongation is complete at the four day stage. There is reason to believe also that in the tip three quarters considerable mitotic activity occurs during the period that most of the cells elongate. Thus the distal cell population is not so homogeneous physiologically during elongation as is that of the basal quarter. Consequently the basal quarter may be expected to show the metabolic correlatives of elongation better than the other quarters.

The data discussed above show clearly that the respiration rate, R.Q., and water content of the developing first leaf of wheat change as the leaf tissue ages. The fact that the R.Q. changes indicates that the relative quantities of material passing along the different pathways of metabolism are changing. In a later paper it will be shown that the concentration of at least one enzyme, namely invertase, changes during ontogeny and that the maximum concentrations of this enzyme occur at the six day stage in the basal quarter. That the maximum concentration of at least one enzyme does not coincide in time with the maximum respiratory activity gives further support to the view that the relative importance of the different metabolic pathways changes during ontogeny. The pronounced changes in the rate of respiration which accompany development show that the over-all activity changes with age as would be expected.

The changes in the gradients of respiration and R.Q. up to about day six are probably a direct result of the fact that the leaf originates from a basal meristem and so the upper quarters are physiologically older than the basal ones. In the later stages of development the anatomical and other associated factors which have been considered in the two preceding papers of this series (3, 4) become the ones which control the respiratory gradient. The water content gradient is probably largely controlled by the anatomical fact that the basal quarter is nearer to its source of water supply than any of the other quarters. This gradient is strikingly consistent throughout ontogeny and under different cultural treatments.

## Acknowledgments

I wish to express my appreciation to Professors G. H. Duff and D. F. Forward for the many valuable suggestions and criticisms which they have made during the course of this study.

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# THE GENUS *TYMPANIS*[1]

By J. Walton Groves[2]


## Abstract

This paper is a taxonomic study of the genus *Tympanis* with a discussion of its history, morphology, conidial relations, parasitism, and distribution. Thirty-six species and one variety are recognized, of which 23 species have been studied in cultures. Sixteen new species are described and one new name is proposed. The conidial states of *Tympanis* species belong in the form genus *Pleurophomella* v. Höhn. In a section on doubtful and excluded species an attempt is made to account for all the *Tympanis* names in the literature.


## Introduction

The genus *Tympanis* includes a group of inoperculate Discomycetes occurring on twigs, branches, or main trunks of woody plants. The morphological features that characterize the genus are relatively clear-cut and include the presence of black or blackish apothecia of very hard consistency, with plectenchymatous tissue and multispored asci, and the occurrence of flask-shaped pycnidia bearing minute conidia.

Although it is easy to recognize the members of the genus, the identification of species is difficult. The primary ascospores are of some value as diagnostic characters when they can be found, and the growth habit is of some assistance, but the only morphological character of much value is the size of the asci. This, combined with cultural studies, has supported the view that the species are, in nature, generally specific to their hosts. The identity of the host is, therefore, considered to be of considerable importance in determining the species of *Tympanis*.

The investigation of this genus was first begun in 1932 at the suggestion of Professor H. S. Jackson, and since then a great deal of material has been collected and as many specimens as possible have been cultured. Recently it has been possible to visit several European herbaria and specimens have been generously loaned by other institutions and individuals. Frequent references have been made in the literature to certain species and groups of species, but no critical and comprehensive study of the genus has been undertaken. It was, therefore, decided to prepare a monographic treatment of all of the known species, based on the knowledge gained during many years study of fresh material and cultures.

## Historical

The genus *Tympanis* was erected by Tode (1790 (41) ) based on the single species *T. saligna*, occurring on *Salix*. None of Tode's material appears to be in existence and it is not possible to identify his species with certainty from the description. However, of the two species of *Tympanis* known to

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occur on *Salix*, one, which is characterized by more or less immersed to slightly erumpent, grayish-pruinose apothecia, and very long asci, appears to be the more common and better known species. This fungus has been identified as *T. saligna* by many authors and it would seem desirable to designate a neotype and fix the concept of this species as the type of the genus. This will stabilize the nomenclature in the sense of long-established usage.

The genus was recognized by Fries (1822 (6) ) who listed eight species including *T. saligna* Tode. Of these eight species, three (*T. saligna*, *T. alnea*, and *T. conspersa*) are still recognized as species of *Tympanis*. Fries thought that the genus was intermediate between the Pyrenomycetes and Discomycetes and that it probably should be placed in the former. Probably he observed the flask-shaped pycnidia of the conidial state and believed that these were young stages which later expanded to form the disks. His generic concept appears to have been based principally on the color, consistency, and presence of the young pyrenomycete-like conidial state rather than on the microscopic characters.

Schweinitz (1832 (36) ) listed nine species of *Tympanis*. His concept of the genus was similar to that of Fries, but only three of the species he included (*T. alnea*, *T. conspersa*, and *T. fasciculata*) are still regarded as species of *Tympanis*.

Wallroth (1833 (44) ) broadened the concept and added a number of diverse forms, listing a total of 15 species. Only three of these (*T. saligna*, *T. aucupariae*, and *T. conspersa*) are still retained in *Tympanis* and the latter two are regarded as synonyms.

The modern concept of the genus really dates from the work of the Tulasnes (1865 (43) ). Although they only described the two species *T. ligustri* and *T. pinastri* in detail, and the latter name is untenable under the International Rules of Nomenclature, the descriptions were so complete and accurate that the Tulasnes were undoubtedly mainly responsible for the relatively stable concept of the genus that was followed by later authors. They emphasized the multispored asci and hard consistency of the apothecia, and clearly traced the relationship between the perfect and imperfect states. They referred to Fries' suggestion that the genus should be placed in the Pyrenomycetes but pointed out that, although the imperfect state resembled a pyrenomycete, its position in the classification should be determined by the perfect state.

Nylander (1868 (24) ) was the first to draw attention to the primary ascospores and describe them.

Saccardo (1889 (34) ) listed 24 species and three varieties, but no critical investigation of the genus was made until Rehm (1889 (30) ) studied 16 species and one variety and added four species that he considered doubtful.

Brefeld (1891 (3) ) described the characteristic growth in culture and the formation of secondary ascospores, but it is difficult to decide which species he studied.

Boudier (1907 (2) ) compiled a list of 20 species, but does not appear to have subjected them to any critical study.

Nannfeldt (1932 (23) ) did not include the genus in his general system. He suggested that it might require a separate family.

Hansbrough (1936 (11) ) made a careful study of a species causing a canker of red pine. This fungus was later identified by Groves and Leach (1949 (10) ) as *T. confusa* Nyl. Hansbrough's work is the most detailed investigation so far made of any single species, but he did not make a comparative study with other species.

Seaver (1951 (38) ) listed six species of *Tympanis*, but apparently had a misconception of the genus. He stated: "The genus *Tympanis* comprises a number of species in which normal ascospores are absent or undeveloped. Ascospores sometimes develop in addition to the spermatoid bodies in which case the species is transferred to some other genus depending upon the nature of the ascospores (see *Durandiella fraxini*) . . ." The presence of both primary and secondary ascospores in *Tympanis* has been known since the work of Nylander (1868 (24) ) and the fact that the spermatoid bodies arise from the true or primary ascospores was established by the work of Brefeld (1891 (3) ) and confirmed by Hansbrough (1936 (11) ). Primary ascospores are present in the type species of the genus, *T. saligna*, and have been observed in all the other species described in this paper except one, and they will undoubtedly be found in that species when more material is examined. Their presence is not considered to be any reason for transferring the species to another genus. Furthermore, it can be stated with confidence from examination and cultural studies of numerous specimens of *Durandiella fraxini* (Schw.) Seav. that the filiform ascospores in this species are not accompanied by spermatoid bodies in the ascus. They do not give rise to secondary ascospores but are discharged as filiform spores. There is a group of similar species with filiform ascospores and possessing the aspect and consistency of *Tympanis*, but which do not produce secondary ascospores, do not bud in culture, and have elongated, subfiliform, *Micropera*-like conidia. These form a natural group for which *Durandiella* is the valid genus and they are all easily distinguished from *Tympanis* species. It can only be concluded from Seaver's description of *Durandiella fraxini*, that he did not distinguish between this species with eight filiform ascospores and *Tympanis columnaris* on the same host genus which has ellipsoid primary ascospores and numerous secondary ascospores. The two fungi have quite different conidial states and the distinctions were clearly elucidated by von Höhnel (1918*b* (19) ). It is unfortunate that such serious misconceptions of these two genera should appear in a work of this nature.

Von Höhnel (1914 (15) ) erected the genus *Pleurophomella* to include conidial states of *Tympanis* species. This genus was based on *P. eumorpha* (Penz. & Sacc.) v. Höhn. and it is evidently the conidial state of one of the conifer-inhabiting species, but it is impossible to decide to which one it belongs. The conidial states of *Tympanis* species had previously been referred to several form genera, such as *Dothiorella* and *Dendrophoma*. If a generic name is needed for these conidial states, *Pleurophomella* v. Höhn. is available, but in

practice it is not a very useful genus because the conidial states of all the *Tympanis* species are so similar morphologically that, unless they are associated with apothecia, it is impossible to identify them.

## Apothecia

The apothecia are usually found breaking through the bark of the host, but may occur rarely on decorticated wood. They may occur singly or in large clusters. In general, this habit of growth is fairly constant, a species either occurs singly or in small clusters of up to five or six, or cespitose in clusters of 10 or more. In shape they are mostly circular or undulate, sessile, narrowed toward the base, and sometimes appear to be substipitate. The color is black, but some species are more or less densely grayish-pruinose. Under moist conditions the hymenium is often greenish. The consistency is very hard and horny when dry, becoming cartilaginous or somewhat rubbery when moist, with the hymenium softer and more fleshy.

The tissue structure is very similar in all the species. It is plectenchymatous, composed of slender, interwoven hyphae embedded in a gelatinous matrix which appears to consist of the thickened walls of the hyphae. The excipulum is only slightly differentiated as an outer rindlike zone, and the subhymenial zone is scarcely if at all differentiated. The paraphyses are slender, often branched, with the tips slightly enlarged and embedded in a brownish, gelatinous matrix forming a distinct epithecium.

The mature asci are multispored, containing hundreds of minute, hyaline spores which are very similar in all of the species, and of no value as diagnostic characters of the species. These are actually secondary spores and may be regarded as conidia. They are, in fact, indistinguishable from the conidia produced in the pycnidial fruiting bodies. The true or primary ascospores, which are usually eight in number, are difficult to find and have never been described in a great many of the species. They are generally indistinct and soon obscured by the secondary ascospores, so that in many mounts they cannot be found.

Brefeld (1891 (3) ) described and illustrated the secondary ascopores as arising by budding from the primary ascospores and my observations would support this view. Primary ascospores are sometimes observed apparently bearing appendages and these have been interpreted as budding secondary spores. An intermediate stage is sometimes observed in which the asci contain a number of corpuscular bodies that are larger than the ultimate secondary ascospores but smaller and more numerous than the primary ascospores. In a few instances these bodies have been observed emerging from broken asci. It is believed that such asci are responsible for reports in the literature of 16- and 24-spored asci. I have found no instance of an ascus containing more than eight primary ascospores.

The primary ascospores fall into three main groups according to their shape. In most of the species they are ovoid or broadly ellipsoid to globose

(somewhat clavate in one species), sometimes with one septum but usually one-celled. In some species they are more or less fusoid and usually two-celled, whereas in others they are more elongated, several-celled, and sometimes muriform. They are of secondary importance in identification of species because of the difficulty of observing them and the fact that some variation in their size and shape frequently occurs in the same species. They are of value in placing the species in one of the three groups mentioned above, but cannot be used with certainty for separating species within these groups.

The asci, themselves, provide the most important characters for separating the species. They are typically cylindric to cylindric-clavate, with a short slender stalk and obtuse thickened apex. In general, the length of the asci varies more than the width, as the width of the mature asci is relatively constant. When the asci are in the primary ascospore stage, and for some time after the secondary ascospores have begun to form, the wall of the ascus is greatly thickened and gelatinized. As the ascus approaches maturity, it increases slightly both in length and width and the wall becomes thinner. If measurements are made on the thick-walled asci, they will vary considerably, whereas in the fully mature, thin-walled ones they are relatively constant. This is an important point when measuring the asci. In some species, notably those of the "conspersa" group, the asci appear to mature slowly and in succession, so that it is often difficult to find many mature asci in a mount.

Hansbrough (1936 (11) ) suggested that the asci should be regarded as physiologically mature when the primary ascospores are formed, but not morphologically mature until the secondary ascospores are formed. However, I have found no evidence that the asci are ever discharged at the primary ascospore stage, so that it is questionable whether they may be regarded as physiologically mature at that time.

## Pycnidia

The genetic connection between the perfect and imperfect states has been established by cultural methods in 23 species. In contrast to *Dermea* (Groves 1946 (9) ), where the conidial fruiting bodies and conidia are of great value in differentiating species, in *Tympanis* they are of slight diagnostic value. In all of the species in which the connection has been established, the conidial fruiting bodies are very similar in form and structure and the conidia are indistinguishable in the different species.

The fruiting bodies are typically small, black or grayish-pruinose, ovoid to cylindric pycnidia. They may be separate or cespitose and frequently arise from the same basal stroma as do the apothecia (Fig. 39). The tissue structure is the same as that of the apothecia. They contain an ovoid cavity opening more or less widely at the top and lined with filiform conidiophores which produce conidia both at the tip and along the sides at the septa (Figs. 76, 77). The conidia are indistinguishable from the secondary ascospores and from the conidia that are budded off the hyphae in culture. None of the species can be recognized by the conidia.

In some species the conidial fruiting bodies are few and inconspicuous, whereas in others they are produced abundantly, sometimes mostly singly and sometimes densely cespitose. These differences of growth habit are of some assistance in the recognition of species but considerable variation may occur and they can only be considered along with the characters of the apothecia.

In the "conspersa" group the conidial fruiting bodies are usually found on the same stroma as the apothecia and are fairly conspicuous. In *T. spermatiospora* and *T. fasciculata* they are also fairly conspicuous, usually cespitose in the former and single in the latter. In many of the species the conidial fruiting bodies are usually few and inconspicuous, but it has been possible to find them in all but three of the species collected in fresh condition. In species of which only scanty dried material has been available, no conidial fruiting bodies were found, but there is no reason to doubt that they will be discovered when fresh material is studied.

## Cultural Studies

In a genus such as this, where the morphological characters are very similar in the different species, the cultural characters are of special interest as a means of distinguishing species. Of the 36 species recognized in this paper, 23 have been studied in culture. Isolations have been made from both ascospores and conidia whenever possible, and also from as many different collections as possible.

Isolations from conidia were made in the following manner. Twigs bearing pycnidia were placed in a moist chamber and left overnight. Usually the conidia appeared as masses or cirrhi on top of the pycnidia, or they would emerge if light pressure was applied to the pycnidium with the point of a needle. The fresh spore masses were transferred to a tube of melted malt extract agar and plates poured with the dilute spore suspension.

The ascospore cultures were made by fastening an apothecium to the lid of a Petri dish and allowing it to discharge spores on to the agar below. No evidence was obtained that the asci ever discharge the primary ascospores. The whole mass of secondary ascospores appears to be discharged simultaneously and they adhere together in a clump. The spores in the clump at first develop a yeastlike colony by budding, and then a few slender, hyaline, immersed hyphae appear at the margin like a fringe. Conidia continue to be budded from these hyphae and the colonies resemble those of *Pullularia pullulans* (DeBary) Berkh., but the spores of the latter are much larger. Later there may be some aerial mycelium produced but as a rule it is scant.

Cultures were grown on 2% malt extract agar and on sterilized twigs of the host. The twig cultures were prepared in the same manner as described by Groves (1946 (9) ) for *Dermea* species. In most species, conidial fruiting bodies are produced readily on both agar and twigs, and in several species apothecia were also produced on the twigs. Where apothecia were produced on the twigs, the asci and primary ascospores have agreed morphologically with those found in nature.

Repeated attempts have been made, especially with the conifer-inhabiting species, to grow these fungi on sterilized twigs of trees other than the original host. Usually these attempts were successful and conidia, and often also apothecia, were produced. However, in each case when isolations were made either from ascospores or conidia, the cultures resembled those from the original host. The evidence suggests that in nature they are specific to certain hosts.

In general, colonies of these fungi on malt extract agar are appressed, more or less sodden, and give the impression that they are contaminated with bacteria. There are differences in growth rate, color, character of the margin, and abundance of conidial fruiting bodies. The color range is not great, mostly shades of gray and brown to yellowish, with the exception of *Tympanis pseudotsugae* which is green. The cultural differences are best observed in cultures three to four weeks old.

Of the 12 species recognized as occurring on conifers, 11 have been studied in culture. Some of these species are readily recognizable on morphological characters alone, whereas others are recognized chiefly on the basis of cultural characters and host specificity.

Although it is realized that in some groups of fungi the value of cultural characters and host substrate as a basis of species differentiation is seriously open to question and sometimes quite useless, nevertheless in other groups these characters are of value. In *Tympanis*, isolations from various collections of different species that can be readily recognized morphologically indicate that they are specific to certain hosts and, in general, have a fairly constant appearance in culture. Therefore, when forms that are morphologically similar have exhibited cultural differences, I have decided to recognize them as species. Some would, no doubt, prefer to regard these "host species" as varieties of a single species and there is much to be said for this point of view. However, it is felt that, in a little known group such as this, it is preferable to emphasize differences in preliminary studies. If more intensive studies subsequently show that these differences cannot be maintained, less taxonomic difficulties are created by placing species in synonymy than by attempting to disentangle forms that have been prematurely placed under the same name. As a result of the cultural studies, it does seem that the forms occurring on different hosts are not the same, and it seems desirable to recognize these differences. Whether they should be regarded as of varietal or specific rank is, in the light of present knowledge, merely a matter of personal opinion.

Among the conifer-inhabiting species, three main groups may be recognized, depending on the primary ascospores. In the first one, the primary ascospores are elongated and several-septate, in the second, fusoid and one- or two-celled, and in the third broadly ellipsoid to subglobose.

In the first group there are only two species, *T. pseudotsugae* and *T. confusa*, and these are clearly distinct morphologically and culturally. *T. pseudotsugae* is the most distinctive species in the genus.

The group with subglobose primary ascospores contains four species which are also fairly readily distinguished, *T. pithya*, *T. piceae*, *T. tsugae*, and *T. truncatula*. Of these *T. pithya* has the largest asci, *T. piceae* has shorter, broad asci, whereas *T. tsugae* and *T. truncatula* both have rather long, slender asci. The latter two might be difficult to separate by means of the asci alone, but the apothecia of *T. tsugae* are smaller and not so strongly erumpent. The cultural characters are also different. *T. truncatula* grows more rapidly, has an indistinct margin, and produces few pycnidia, whereas *T. tsugae* grows more slowly, has a clear cut margin, is a more opaque colony, and produces abundant small pycnidia.

The group with fusoid primary ascospores is the most difficult one. *T. laricina* can be fairly readily separated by the larger asci but the remainder, including *T. hypopodia*, *T. abietina*, *T. piceina*, *T. hansbroughiana*, and *T. juniperina*, are nearly identical morphologically. Numerous isolations of *T. hypopodia* and *T. abietina* have proved to be quite distinct culturally. *T. abietina* grows slowly, producing considerable brownish aerial mycelium, and the colony has a raised abrupt margin and pycnidia are abundant. In *T. hypopodia* the colony grows more rapidly with an indistinct, appressed margin, very little aerial mycelium, and few or no pycnidia.

In one instance a pile of brush containing branches of both *Pinus* and *Abies* was found to have *Tympanis* on both, but cultural and morphological studies revealed that *T. abietina* was the species on the *Abies* and *T. pithya* the species on *Pinus*.

The only specimen of *T. hansbroughiana* that has been cultured was collected by J. R. Hansbrough at Petersham, Mass. Since this is out of the normal range of *Pseudotsuga*, it might have been expected that one of the other species of the region was occurring on it rather than a different one. Nevertheless, one western collection agreeing morphologically with the eastern one has been seen and, since it does not resemble any of the known eastern species in culture, it seems preferable to regard it as a distinct species.

*T. juniperina* is the only species of this group which has not been studied in culture and is known only from examination of the type in Kew Herbarium. It is definitely of the "hypopodia" group and can scarcely be separated morphologically from the other species in the group, but in order to keep the treatment consistent it is retained as a distinct species.

Cultural studies have been much less complete among the species inhabiting broad-leaved hosts. It has only been possible to study in culture 12 of the 24 species recognized. However, as far as they have gone, the cultural studies have indicated here also that the species are generally specific to their hosts. For example, the cultural studies have been very helpful in determining the identity of the species on *Alnus*. This species is typically black, glabrous, and densely cespitose, but is sometimes grayish-pruinose, and sometimes a form is found in which the apothecia are single, more or less elongated, and usually larger than those of the cespitose form. Rehm (1889 (30) )

described this form as a distinct species and later (1912 (31) ) reduced it to a variety. Some authors have referred the pruinose cespitose form to *T. conspersa*, a form of similar appearance occurring on *Malus*. All of these forms seem to be identical in microscopic characters. In culture all the *Alnus* forms were very similar and much more like each other than like the form on *Malus*. It has, therefore, been decided to consider all the *Alnus* forms as one species, and to recognize the form with single, elongated apothecia as a variety.

## Parasitism

The host specificity exhibited by the various species would suggest that they are at least weakly parasitic. Hansbrough (1936 (11) ) has shown that at least one species, *T. confusa* Nyl., produces cankers on the host and can be of considerable economic importance. No other species has been subjected to a critical study from this standpoint, and it is evident that this species only became of importance under a special set of environmental factors that affected the host adversely. In general, the species of *Tympanis* may be regarded as of minor importance as pathogens.

## Distribution

From the evidence available, *Tympanis* appears to be a genus of the north temperature zone. Many of the more commonly collected species, such as *T. conspersa*, *T. alnea*, *T. spermatiospora*, *T. pithya*, *T. confusa*, and *T. hypopodia*, are present in both North America and Europe. On the other hand certain species, such as *T. columnaris* and *T. syringae*, appear to be confined to Europe, whereas others that are fairly common in North America, such as *T. fasciculata*, are not known in Europe.

The distribution may, in many cases, be limited only by the distribution of the host, but scarcely enough collecting of the various species has been done, and they are too poorly known to permit of any such generalization at present.

## Taxonomy

The order in which the species are listed below should not be construed as representing any phylogenetic arrangement. Owing to the great similarity in morphology of so many characters in the various species any phylogenetic grouping is very difficult.

Undoubtedly the most striking species in the genus is *T. pseudotsugae*. The large size of the apothecia, asci, and primary ascospores is very distinctive and cultures of this species are more mycelial in character than in any other species investigated. Close to this species is *T. confusa* by reason of the similar shape of the primary ascospores.

The remaining species fall into two groups based on the shape of the primary ascospores. In the one group the primary ascospores are broadly ellipsoid to ovoid or subglobose and in the other they are fusoid. The bulk of the species fall into the group with subglobose spores and here are found species

with small asci, intermediate asci, or large asci. A group of species centering around *T. conspersa* and including *T. alnea*, *T. amelanchieris*, *T. prunicola*, *T. magnoliae*, and *T. rhoina*, show a number of characters in common, including a cespitose habit of growth, a tendency to be grayish-pruinose, and large asci relatively few of which are usually found in the fully mature condition in any one mount. Close to these are *T. saligna* and *T. myricariae* which differ in the usually separate apothecia and weakly erumpent habit of growth.

Of the species with fusoid primary ascospores, only one occurs on broad-leaved hosts and in it they are more clavate than fusoid, and a closely related group of six occurs on conifers. All of these species have small asci.

The size of the asci might conceivably be of some evolutionary significance in a group such as this where the ascus becomes filled with many secondary spores. Presumably the larger asci would contain more spores and thus possess some biological advantage.

Two species, *Retinocyclus olivaceus* Fckl. and *Corynella atrovirens* (Pers.) Boud., have been included in the key below because they are likely to be looked for in this genus, although they are not regarded as true *Tympanis* species.

The following code letters are used in referring to herbaria in which the specimens cited are deposited: BM—British Museum; DAOM—Mycological Herbarium, Division of Botany and Plant Pathology, Science Service, Ottawa; FH—Farlow Herbarium; JRH—J. R. Hansbrough; JWG—J. W. Groves; K—Kew Herbarium; LEW—L. E. Wehmeyer; M—University of Michigan; NYBG—New York Botanical Garden; P—Mus. d'hist. Nat., Paris; S—Botanical Museum, Stockholm; TRT—University of Toronto; UIFP—University of Idaho, Forest Pathology; UPS—Institute of Systematic Botany, Uppsala; WBC—Wm. Bridge Cooke; WLW—W. L. White; ZT—Eidg. Techn. Hochsch., Zurich.

## Generic Diagnosis

*Tympanis* Tode ex Fr. Syst. Myc. 2 : 172. 1822.

Apothecia erumpent, separate or cespitose, circular to undulate, sessile or narrowed below to substipitate, black or blackish, glabrous or pruinose, hard, horny in consistency, becoming cartilaginous when moist with the hymenium more fleshy; tissue plectenchymatous with slightly differentiated rindlike excipulum; asci cylindric to cylindric-clavate, obtuse, at first four- to eight-spored, finally multispored; primary ascospores subglobose to ovoid, or fusoid, one- to several-celled, secondary ascospores minute, cylindric to allantoid, very numerous; paraphyses hyaline, filiform, the tips forming an epithecium; conidial fruiting bodies ovoid to subcylindric or conic, separate or cespitose, glabrous or pruinose, black or blackish, similar in consistency and tissue structure to the apothecia, conidiophores filiform, septate, bearing conidia at the tip and along the sides, conidia similar to the secondary ascospores.

TYPE SPECIES: *Tympanis saligna* Tode ex Fr.

## Key to the Species

1. Occurring on coniferous hosts ..... 2
1. Occurring on frondose hosts ..... 15

2. Secondary ascospores globose, on resin ..... *Retinocyclus olivaceus*[1]
2. Secondary ascospores cylindric to allantoid ..... 3

3. Asci exceeding 175μ in length, on *Pseudotsuga* ..... 2. *T. pseudotsugae*
3. Asci shorter ..... 4

4. Asci mostly exceeding 100μ in length ..... 5
4. Asci mostly less than 100μ in length ..... 10

5. Primary ascospores elongated or fusoid ..... 6
5. Primary ascospores subglobose ..... 7

6. Primary ascospores fusiform, 2-celled, asci mostly less than 110μ in length, on *Larix* ..... 7. *T. laricina*
6. Primary ascospores elongate-fusiform, several-celled, asci more than 110μ in length, on *Pinus* ..... 1. *T. confusa*

7. Mature asci mostly exceeding 15μ in diameter, on *Pinus* ..... 3. *T. pithya*
7. Mature asci less than 15μ in diameter ..... 8

8. Apothecia scarcely exceeding 0.8 mm. in diameter, not strongly erumpent, asci mostly 12-14μ in diameter, on *Tsuga* ..... 6. *T. tsugae*
8. Apothecia reaching 1 mm. or more in diameter, not on *Tsuga* ..... 9

9. Conidial fruiting bodies conspicuous, cespitose on the same stroma as the apothecia, asci mostly 14-16μ in diameter, on *Picea* ..... 4. *T. piceae*
9. Conidial fruiting bodies usually scattered, singly to subcespitose, rarely on the same stroma as the apothecia, asci mostly 12-15μ in diameter, on *Abies* ... 5. *T. truncatula*

10. Mature asci reaching 14μ or more in diameter, on *Larix* ..... 7. *T. laricina*
10. Mature asci rarely exceeding 13μ in diameter ..... 11

11. Mature asci mostly 10-11μ in diameter, on *Juniperus* ..... 12. *T. juniperina*
11. Mature asci mostly 11-13μ in diameter ..... 12

12. Asci mostly less than 90μ in length, primary ascospores 5-8μ in length on *Picea* ..... 10. *T. piceina*
12. Asci frequently more than 90μ in length, primary ascospores 7-10μ in length ..... 13

13. Apothecia scattered, on *Pseudotsuga* ..... 11. *T. hansbroughiana*
13. Apothecia usually densely gregarious, not on *Pseudotsuga* .....

14. On *Pinus* ..... 8. *T. hypopodia* 14
14. On *Abies* ..... 9. *T. abietina*

15. Mature asci mostly exceeding 18μ in diameter ..... 16
15. Mature asci mostly less than 18μ in diameter ..... 26

16. Mature asci frequently exceeding 165μ in length ..... 17
16. Mature asci less than 165μ in length ..... 20

[1] *See Tympanis olivacea in Doubtful and Excluded Species.*

## Key to the Species—*Continued*

17. Apothecia single or rarely subcespitose ..... 18
17. Apothecia in clusters of more than six ..... 19

18. Apothecia somewhat hysterioid, black, strongly erumpent, on *Alnus* ..... 16. *T. alnea* v. *hysterioides*
18. Apothecia circular, grayish-pruinose, immersed to slightly erumpent, on *Salix* ..... 13. *T. saligna*

19. Apothecia densely white-pruinose, rarely black and glabrous, on *Malus* or *Sorbus* ..... 15. *T. conspersa*
19. Apothecia black, glabrous, sometimes grayish-pruinose, on *Alnus* or *Betula* ..... 16. *T. alnea*

20. Asci mostly less than 110$\mu$ in length, on *Hydrangea* ..... 26. *T. hydrangeae*
20. Asci mostly exceeding 110$\mu$ ..... 21

21. Asci mostly less than 130$\mu$ in length ..... 22
21. Asci mostly more than 130$\mu$ in length ..... 23

22. Apothecia strongly erumpent, usually exceeding 0.3 mm. in height, conidial fruiting bodies usually conspicuous, on *Viburnum* ..... 25. *T. fasciculata*
22. Apothecia not strongly erumpent, usually less than 0.3 mm. in height, conidial fruiting bodies few or not present, on *Syringa* ..... *T. syringae*

23. Apothecia cespitose, frequently more than six in a cluster, usually grayish-pruinose .. 24
23. Apothecia separate to subcespitose, usually less than six in a cluster, black, glabrous .. 25

24. Asci mostly exceeding 145$\mu$ in length, on *Magnolia* ..... 19. *T. magnoliae*
24. Asci mostly less than 145$\mu$ in length but some up to 175$\mu$, on *Prunus* .. 17. *T. prunicola*

25. Mature asci mostly exceeding 145$\mu$ in length, on *Diospyros* ..... 21. *T. diospyri*
25. Mature asci mostly less than 145$\mu$ in length, on *Oxydendrum* ..... 22. *T. oxydendri*

26. Mature asci mostly 15-18$\mu$ in diameter ..... 27
26. Mature asci mostly less than 14$\mu$ in diameter ..... 35

27. Mature asci mostly less than 115$\mu$ in length ..... 28
27. Mature asci mostly more than 115$\mu$ in length ..... 30

28. Apothecia strongly cespitose with up to 15 in a cluster, on *Betula* ... 31. *T. mutata*
28. Apothecia separate to subcespitose ..... 29

29. Apothecia not strongly erumpent, scarcely exceeding 0.3 mm. in height, on *Forsythia* ..... 27. *T. forsythiae*
29. Apothecia strongly erumpent, up to 1 mm. in height, on *Acer* ..... 29. *T. acericola*

30. Asci mostly exceeding 150$\mu$ in length, on *Myricaria* ..... 14. *T. myricariae*
30. Asci mostly less than 150$\mu$ in length ..... 31

31. Apothecia not strongly erumpent, scarcely exceeding 0.3 mm. in height, usually single, on *Ligustrum* ..... 24. *T. ligustri*
31. Apothecia usually exceeding 0.5 mm. in height, more cespitose ..... 32

32. Apothecia more or less grayish-pruinose ..... 33
32. Apothecia black, glabrous ..... 34

33. On *Amelanchier* ..... 18. *T. amelanchieris*
33. On *Rhus* ..... 20. *T. rhoina*

## Key to the Species—*Concluded*

34. Primary ascospores subglobose, 4.5 × 3.5-4.5μ, *on Fraxinus*.....30. *T. columnaris*
34. Primary ascospores ellipsoid 5-6 × 2-3μ, on *Salix*.....28. *T. salicina*

35. Asci less than 60μ in length (?), on *Cephalanthus*.....36. *T. cephalanthi*
35. Asci exceeding 60μ in length..... 36

36. Asci mostly 70-90μ in length..... 37
36. Asci mostly 85-100μ or more in length..... 38

37. Asci rarely exceeding 12μ in diameter, on *Malus*.....34. *T. malicola*
37. Mature asci frequently exceeding 12μ in diameter, on *Populus*..32. *T. spermatiospora*

38. Primary ascospores subglobose, on *Sorbus*.....33. *T. sorbi*
38. Primary ascospores subclavate, one- or two-celled, on *Fagus*.....35. *T. rehmiana*
38. Primary ascospores elongated, several-celled, on various hosts..*Corynella atrovirens*[1]

[1] *See Tympanis atrovirens in Doubtful and Excluded Species.*

1. TYMPANIS CONFUSA Nyl. Obs. Pez. Fenn. p. 69. 1868. (Figs. 1, 47)
*Tympanis amphiboloides* var. *hypopodiza* Nyl. Obs. Pez. Fenn. p. 71. 1868.
*Scleroderris hypopodiza* Sacc. Syll. Fung. 8 : 597. 1889.

Apothecia erumpent, gregarious, separate or cespitose, usually with less than six in a cluster but occasionally in large clusters of 15 or more, circular or slightly undulate, sessile to substipitate, narrowed below, 0.5-1.5 mm. in diameter, 0.4-2.0 mm. in height, black, glabrous, hard, horny in consistency, becoming more cartilaginous-fleshy when moist; hymenium concave to plane, dull black or shiny black, more fleshy than the excipulum, at first with a thick, somewhat infolded margin which later may disappear; tissue of the hypothecium plectenchymatous, composed of interwoven, ascending, brownish to nearly hyaline hyphae about 1-2μ in diameter, with the walls greatly thickened and gelatinized, becoming thicker and darker at the outside forming a rindlike excipulum, subhymenium not clearly differentiated; asci cylindric to cylindric-clavate, short-stalked, at first with the walls thickened and gelatinized, becoming thinner with maturity, at first 1-4-(8)-spored, finally multispored, (80)-85-120-(150) × (10)-12-16-(21)μ; primary ascospores hyaline, elongate-fusoid to clavate-fusoid, one- to several-celled, occasionally muriform, irregularly biseriate to uniseriate, 13-20 × 2-4μ; secondary ascospores hyaline, cylindric to allantoid, one-celled, 2.0-3.0 × 1.0-1.5μ; paraphyses hyaline, filiform, septate, simple or branched, 1.5-2.0μ in diameter, the tips slightly swollen to 3.0μ and embedded in a gelatinous matrix forming a brownish epithecium.

Conidial fruiting bodies erumpent, scattered to gregarious, separate or cespitose, black, glabrous, globose to ovoid, sessile, 0.2-0.3 mm. in diameter, or sometimes on long, branched, cylindric or irregular stalks 0.1-0.3 mm. in diameter and up to 2.0 mm. in height, consistency similar to the apothecia; tissue plectenchymatous, similar to that of the apothecia, in the upper part containing a globose to ovoid cavity lined with conidiophores and tearing open at the top; conidiophores hyaline, filiform, septate, simple, about

25-60 × 2.0-2.5$\mu$; conidia hyaline, one-celled, cylindric to allantoid, borne at the tip and along the sides of the conidiophore, 2.0-4.0 × 1.0-1.5$\mu$.

*Host:* *Pinus resinosa* Solander ex Aiton, *P. strobus* L., *P. albicaulis* Engelm., *P. monticola* Dougl., *P. contorta* Dougl., *P. sylvestris* L., *Pinus* spp.

*Type:* Fries Sclerom. Suec. *336* (as *Patellaria atrata* $\beta$.).

*Exsiccati:* Karst. Fung. Fenn. *753* (as *T. amphiboloides* var. *hypopodiza*).

*Specimens Examined:*

CANADA: **Ontario:** Petawawa Forest Experiment Station, JWG *688*.

UNITED STATES: **Connecticut:** Woodbridge, JRH *1126*, JWG *359*; JRH *1134*, JWG *361*; JRH *1136*, JWG *362*; JRH *1509*, JWG *373*; Branford, JRH *1131*, JWG *360*; Windsor, JRH *1120*, JWG *357*; JRH *1108*, JWG *348*; JRH *1109*, JWG *349*; Prospect, JRH *1110*, JWG *350*; Bethany, JRH *1111*, JWG *351*; Hamden, JWG *878*. **New York:** Alder Creek, JRH *1544*, JWG *400*; JRH *1543*, JWG *393*; Canadice, JRH *1539*, JWG *389*; JRH *1540*, JWG *390*; Olive, JRH *1541*, JWG *391*. **Idaho:** Bonner Co., UIFP *2000B*, JWG *756*. **Oregon:** Rhododendron, JRH *1117*, JWG *355*; JRH *1118*, JWG *356*.

EUROPE: **Finland:** Mustiala, UPS (as *T. pithya* Karst.); UPS (as *T. amphiboloides* Nyl.). **Denmark**: Rudeskov, Zealand, leg. O. Rostrup 26.4.91 (as *T. amphiboloides* Nyl.) JRH.

This species was discussed by Groves and Leach (1949 (10) ) and studied in considerable detail by Hansbrough (1936 (11) ). It was described by Nylander (1868 (24) ) based on the specimen in Fries Sclerom. Suec. *336* and labeled *Patellaria atrata* $\beta$. Nylander pointed out that this was obviously a misdetermination since the fungus was a *Tympanis*. Examination of a specimen of this exsiccatum kindly loaned by Miss Cash, disclosed a fungus agreeing closely with Nylander's description with the exception that the asci were not as broad as he stated. Most of the multispored asci in this specimen were 16-18$\mu$ in diameter with an occasional one up to 21$\mu$, whereas Nylander gave the diameter as 24-25$\mu$ for the multispored asci and 16-18$\mu$ for the immature eight-spored asci.

In Hansbrough's material the mature, multispored asci are mostly 14-16$\mu$ in diameter but the primary ascospores appear to be the same. Of the other European specimens cited above, the Rostrup specimen and Karsten specimen

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PLATE I

FIGS. 1. *T. confusa*, apothecia, herb. JWG *756*.

2. *T. laricina*, apothecia, herb. JWG *747*.

3. *T. hansbroughiana*, apothecia, type.

4. *T. piceae*, apothecia and pycnidia, type.

5. *T. hypopodia*, apothecia, herb. JWG *879*.

FIGS. 6. *T. truncatula*, apothecia, herb. JWG *490*.

7. *T. tsugae*, apothecia, type.

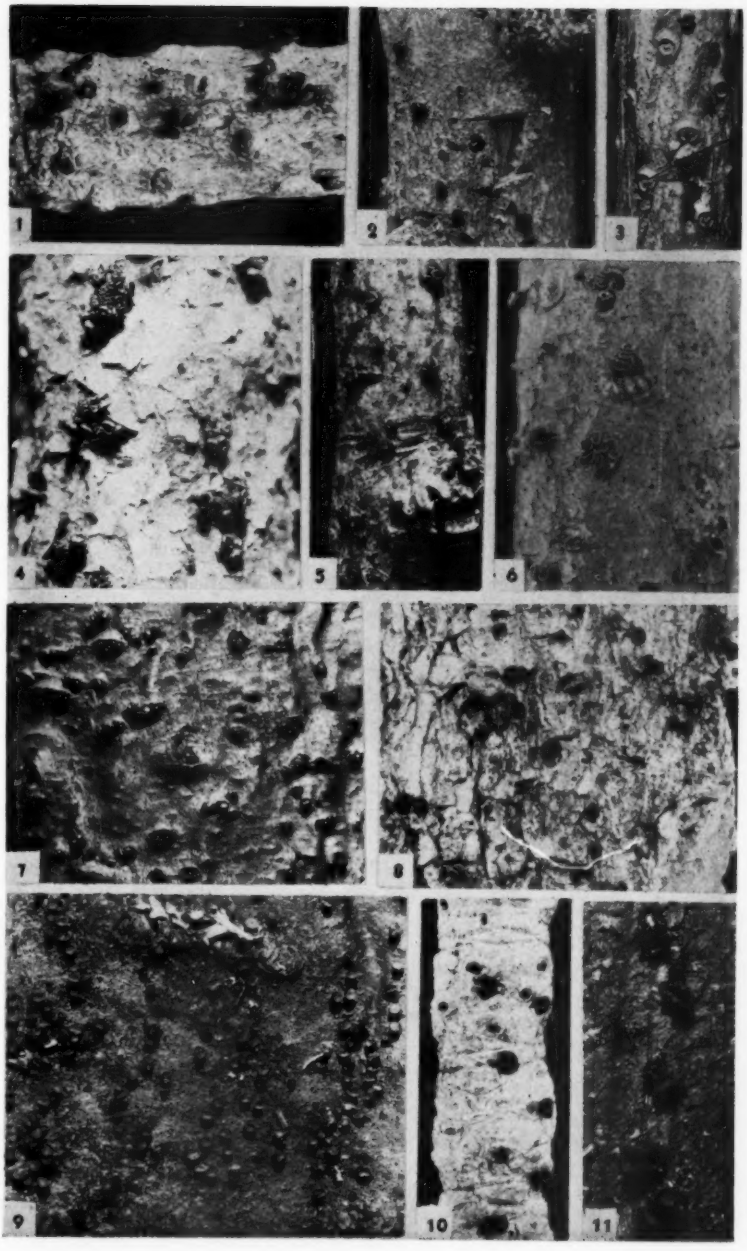
8. *T. piceina*, apothecia, type.

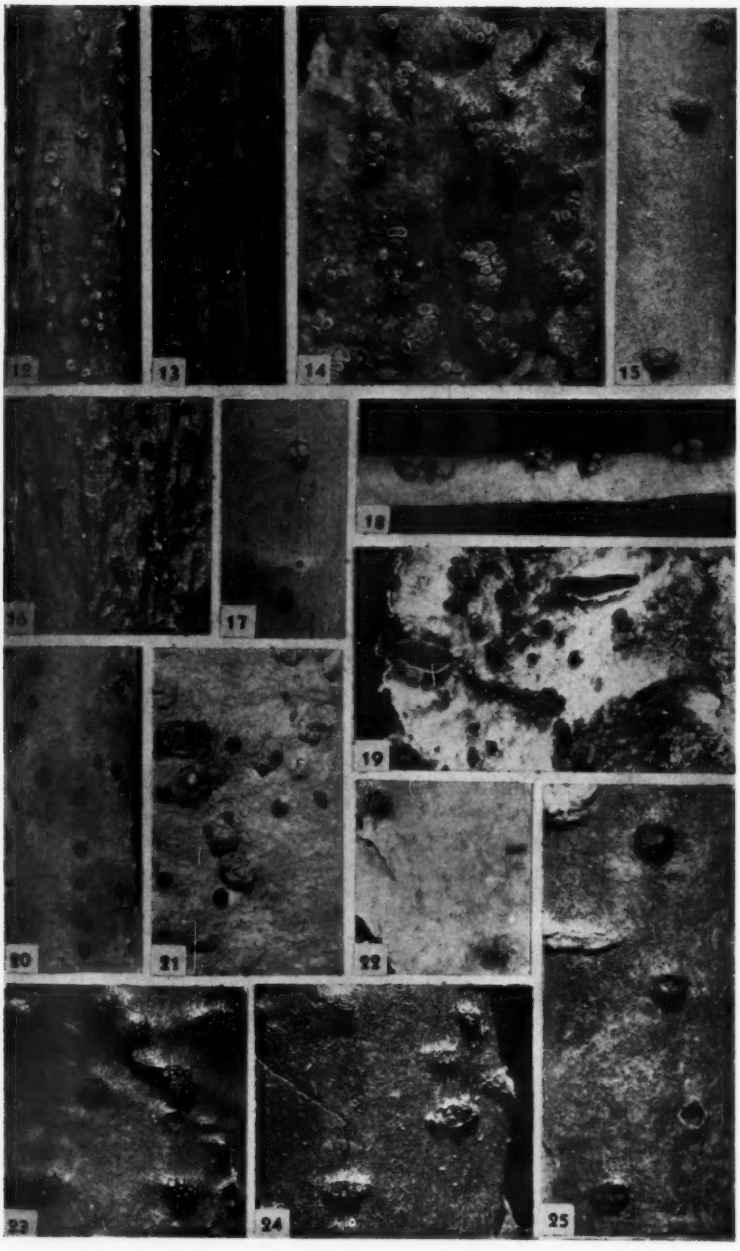
9. *T. abietina*, apothecia, type.

10. *T. pithya*, apothecia, herb. JWG *833*.

11. *T. pseudotsugae*, apothecia, herb. JWG *353*.

PLATE I

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labeled *T. pithya* agreed more closely with Hansbrough's material, whereas the Karsten specimen labeled *T. amphiboloides* and Karst. Fung. Fenn. *753* had the broader asci.

No cultures have been studied from a specimen with these broader asci and it is possible that there may be two species here, in which the primary ascospores are indistinguishable. Examination of mounts illustrating the extremes of ascus size would certainly give that impression. Some mounts from Hansbrough's collections have asci almost down to the size range of *T. hypopodia*, but they are distinct from this species in the elongated, septate, primary ascospores. Other collections with these elongated primary ascospores vary in size to a decided overlap with those of *T. confusa*.

If it should become necessary to recognize two species, probably an ascus width of about 16$\mu$ would be the most convenient dividing line. Those with asci mostly over 16$\mu$ would include the type of *T. confusa* and the two other Karsten specimens cited above, whereas all the other specimens cited have asci mostly less than 16$\mu$ in diameter. These could probably be referred to *Tympanis amphiboloides* var. *hypopodiza* which was described by Nylander (1868 (24) ) as having primary ascospores 18-23 × 3.5-4.0$\mu$ and multispored asci about 16$\mu$ in diameter. It would be necessary to raise this form from varietal to specific rank and give it a new combination in *Tympanis*.

However, in view of the lack of cultural data and the overlap in ascus size that is found on examination of a large series of specimens, it has been decided to lay the most weight on the character of the primary ascospores and to refer any collection with elongated, septate primary ascospores on pine to *T. confusa*.

2. **Tympanis pseudotsugae** sp. nov. (Figs. 11, 40)

Apotheciis erumpentibus, gregariis, solitariis vel caespitosis, sessilibus vel substipitatis, versus basim attenuatis, orbicularibus vel elongatis, undulatis, 1-4 mm. diam., 0.5-3.0 mm. altis, atris, duris, corneis in sicco, cartilagineis in humido; hymenio concavo vel plano, atro, carnoso in humido, marginato; hypothecio plectenchymato; ascis cylindraceis, breve stipitatis, primo

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PLATE II

FIGS. 12. *T. saligna*, apothecia, Krieg. Fung. Sax. *2230* in herb. TRT.

13. *T. myricariae*, apothecia, type, in herb. Bot. Mus. Stockholm.

14. *T. conspersa*, apothecia, herb. JWG *431*.

15. *T. prunicola*, apothecia, herb. JWG *258*.

16. *T. malicola*, apothecia, herb. JWG *363*.

17. *T. rhoina*, apothecia, type, in herb. Farlow.

18. *T. amelanchieris*, apothecia, type.

FIGS. 19. *T. mutata*, apothecia, in herb. Bot. Mus. Stockholm.

20. *T. ligustri*, apothecia, in herb. Farlow ex Barbey-Boissier *1138*.

21. *T. forsythiae*, apothecia, type, in herb. Farlow.

22. *T. magnoliae*, apothecia, type, in herb. Farlow (as *T. viridans*).

23. *T. alnea*, apothecia, black glabrous form, herb. JWG *105*.

24. *T. alnea*, grayish-pruinose form, herb. JWG *240*.

25. *T. alnea* var. *hysterioides*, apothecia, herb. JWG *596*.

octosporis, dein multisporis, (175)-190-240-(260) × (18)-20-23-(25)$\mu$; ascosporis primariis hyalinis, ellipsoideo-fusiformibus, multiseptatis vel muriformibus, 15-25 × 4-6$\mu$; ascosporis secundariis hyalinis, cylindricis vel allantoideis, unicellularis, 2.0-4.0 × 1.0-1.5$\mu$; paraphysibus hyalinis, filiformibus, septatis, simplicibus vel ramosis, 2.0-2.5$\mu$ diam., ad apicem leviter incrassatis agglutinatisque, epithecium fuscum formantibus.

Hab.: *Pseudotsuga taxifolia* (Poir.) Britton

Apothecia erumpent, gregarious, separate or cespitose in clusters of up to 10, sessile to substipitate, narrowed below, circular to slightly elongated or undulate, 1-4 mm. in diameter, 0.5-3 mm. in height, black, sometimes slightly greenish pulverulent, glabrous, hard, horny in consistency, becoming more cartilaginous when moist; hymenium concave to plane, black or greenish when moist, more fleshy than the excipulum, at first with a thick, raised, more or less infolded margin which later may disappear; tissue of the hypothecium plectenchymatous, composed of interwoven, ascending, brownish to nearly hyaline hyphae about 3.0-5.0$\mu$ in diameter, with the walls thickened and gelatinized, at the outside becoming thicker and darker forming a rindlike excipulum; asci cylindric, narrowed below to a short stalk, obtuse at the apex, at first with the walls thickened and gelatinized, becoming thinner with maturity, at first eight-spored, becoming multispored, (175)-190-240-(260) × (18)-20-23-(25)$\mu$; primary ascospores hyaline, ellipsoid-fusiform, several-septate to muriform, uniseriate, 15-25 × 4-6$\mu$; secondary ascospores hyaline, one-celled, cylindric to allantoid, 2.0-4.0 × 1.0-1.5$\mu$; paraphyses hyaline, filiform, septate, simple or branched, 1.5-2.0$\mu$ in diameter, the tips slightly swollen and embedded in a brownish gelatinous matrix, forming an epithecium.

Conidial fruiting bodies erumpent, separate or cespitose, sometimes arising from the same stroma as the apothecia, black, cylindric to cylindric-conic, 0.3-0.7 mm. in diameter, 0.5-1.0 mm. in height, consistency and tissue structure similar to the apothecia, containing a single globose to ovoid cavity, opening at the top; conidiophores lining the cavity, hyaline, filiform, septate, simple, not observed branching, 15-45 × 1.5-2.0$\mu$; conidia hyaline, one-

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PLATE III

FIGS. 26. *T. oxydendri*, apothecia, N. Amer. Fung. *3043*.

27. *T. syringae*, apothecia, in herb. Farlow ex Barbey-Boissier *1100*.

28. *T. hydrangeae*, apothecia, type, in herb. Univ. Mich.

29. *T. sorbi*, apothecia, type.

30. *T. salicina*, apothecia, herb. JWG *588*.

31. *T. acericola*, apothecia, herb. JWG *796*.

32. *T. fasciculata*, apothecia, herb. JWG *865*.

FIGS. 33. *T. cephalanthi*, pycnidia, in herb. J. Dearness.

34. *T. columnaris*, apothecia, in herb. NYBG.

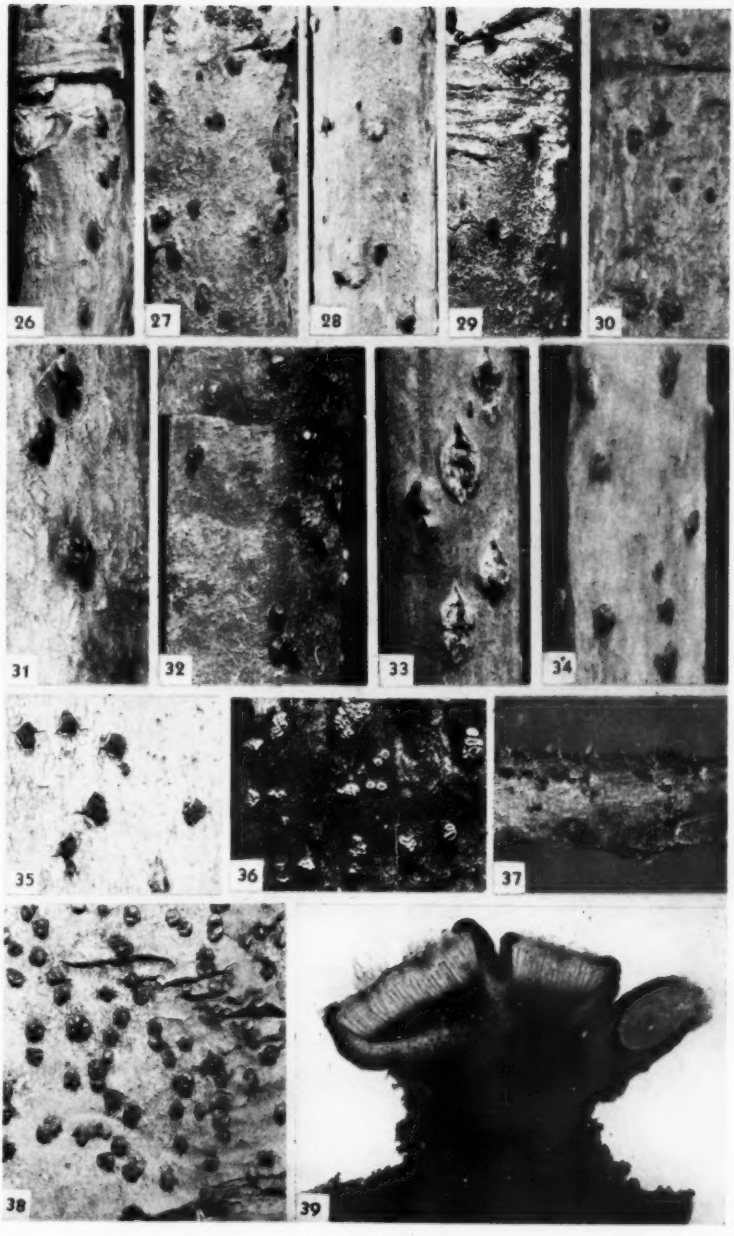
35. *T. diospyri*, apothecia, type.

36. *T. conspersa*, pycnidia, herb. JWG *431*.

37. *T. fasciculata*, pycnidia, herb. JWG *238*.

38. *T. spermatiospora*, apothecia, herb. JWG *50*.

39. *T. fasciculata*, section of apothecia and pycnidium arising from same stroma.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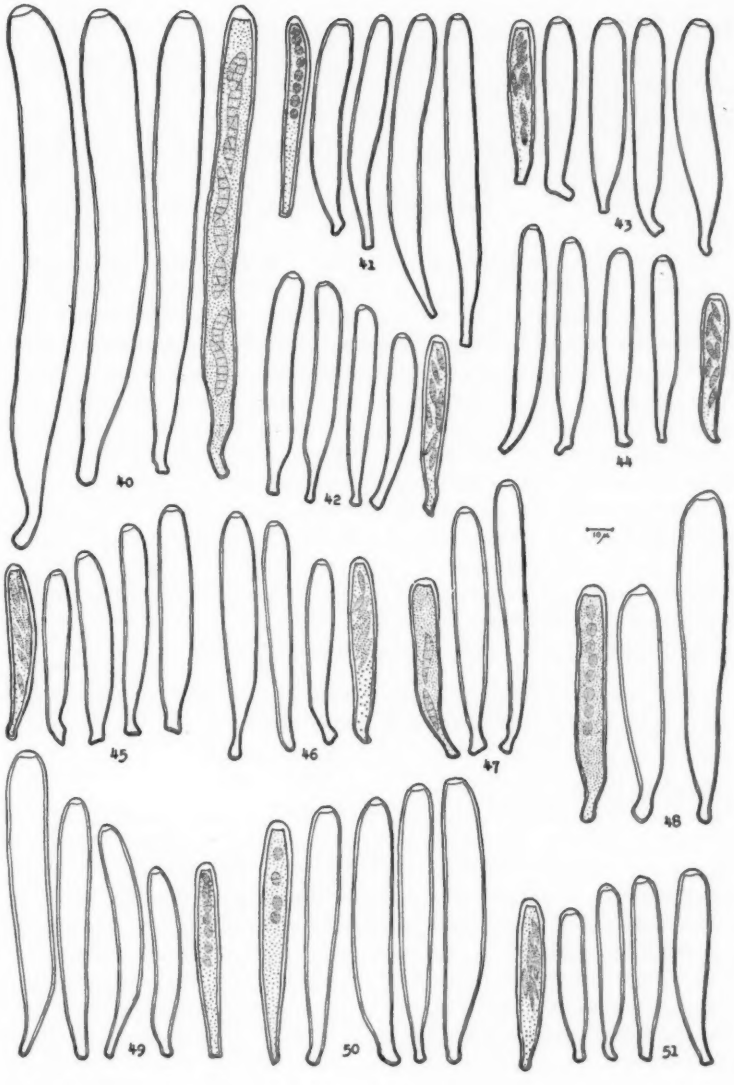



FIGS. 40-51. Drawings of asci and primary ascospores.

FIGS. 40. *T. pseudotsugae.* 41. *T. truncatula.* 42. *T. abietina.* 43. *T. laricina.* 44. *T. juniperina.* 45. *T. hansbroughiana.* 46. *T. hypopodia.* 47. *T. confusa.* 48. *T. pithya.* 49. *T. tsugae.* 50. *T. piceae.* 51. *T. piceina.*

celled, cylindric to allantoid, 2.0-4.0 × 1.0-1.5$\mu$ borne at the tip and along the sides of the conidiophore.

*Host:* *Pseudotsuga taxifolia* (Poir.) Britton

*Type:* DAOM *23807*. Trinidad, California. Coll. H. E. Parks, June, 1939. Co-type JWG *657*.

*Specimens Examined:*

CANADA: **British Columbia:** Owl Creek, JRH *1115*, JWG *353*.

UNITED STATES: **Oregon:** Takilusa, in Herb. Univ. Mich. as *Cenangium leptospermum* B. & C. **California:** Del Norte Co., JWG *637*, H. E. Parks *6212A*.

This is, perhaps, the most distinctive species in the genus. It is remarkable for the large size of the apothecia, the asci, and the primary ascospores. It was first encountered among some of the Hansbrough collections. Later a specimen was received from Miss E. K. Cash that had been collected by H. E. Parks who very kindly supplied fresh material for cultural studies.

The cultures of this species are also very distinctive in appearance. They grow very slowly on malt extract agar, reaching a diameter of 1.5-2.0 cm. in three weeks. They are at first colorless or whitish, but show less tendency to bud conidia from the hyphae than other *Tympanis* species and soon develop a greenish or yellowish-green, velvety, aerial mycelium. They produce a brown discoloration in the medium surrounding the colony and, beyond the brown zone, an indistinct, pale purplish zone. The conidial fruiting bodies are similar to those of other species of *Tympanis*. A culture isolated from a green stain in *Pseudotsuga* in British Columbia, and received from Dr. C. W. Fritz in February, 1940, proved to be this species.

3. TYMPANIS PITHYA (Karst.) Karst. Hedw. 10 : 58. 1871. (Not *T. pithya* Sacc. Syll. 8 : 583. 1889.) (Figs. 10, 48, 77)

*Patellaria pithya* Karst. Fung. Fenn. No. 661. 1867.

*Cenangella pithya* Sacc. Syll. Fung. 8 : 588. 1889.

Apothecia erumpent, gregarious, separate or cespitose, usually in clusters of less than six, circular to strongly undulate, sessile, narrowed below, black, glabrous or occasionally slightly grayish-pruinose, 0.5-1.0 mm. in diameter, 0.3-0.7 mm. in height, hard, horny in consistency, becoming more cartilaginous-fleshy when moist; hymenium concave to plane, dull black or shiny black, more fleshy than the excipulum, at first with a thick, somewhat infolded margin which later may disappear; tissue of the hypothecium plectenchymatous, composed of interwoven, ascending, brownish to nearly hyaline hyphae about 1-2$\mu$ in diameter, with the walls greatly thickened and gelatinized, becoming darker and thicker at the outside forming a rindlike excipulum, subhymenium not clearly differentiated; asci cylindric to cylindric-clavate, short-stalked, at first with the walls thickened and gelatinized, becoming thinner with maturity, at first eight-spored, finally multispored, (80)-95-140-(165) × (11)-14-18-(22)$\mu$; primary ascospores hyaline, broadly ellipsoid to subglobose, one- or two-celled, uniseriate, 5-8 × 3-5$\mu$; secondary ascospores

hyaline, cylindric to allantoid, one-celled, 2.0-3.0 × 1.0-1.5μ; paraphyses hyaline, filiform, septate, simple or branched, 1.5-2.0μ in diameter, the tips slightly swollen to 3.0μ and embedded in a gelatinous matrix forming a brownish epithecium.

Conidial fruiting bodies erumpent, gregarious to scattered, separate or cespitose, black, glabrous, globose or more or less ovoid, sometimes laterally confluent, 0.2-0.3 mm. in diameter, 0.2-0.4 mm. in height, similar in consistency to the apothecia; tissue plectenchymatous, similar to that of the apothecia, in the upper part containing an ovoid to slightly elongated cavity, lined with conidiophores and tearing open at the top; conidiophores hyaline, filiform, septate, simple, about 25-80 × 2.0-2.5μ; conidia hyaline, one-celled, cylindric to allantoid, borne at the tip and along the sides of the conidiophore, 2.0-4.0 × 1.0-1.5μ.

*Host:* *Pinus strobus* L., *P. albicaulis* Engelm., *P. monticola* Dougl., *P. resinosa* Solander ex Aiton, *Pinus* spp.

*Type:* Karst. Fung. Fenn. *661*.

*Specimens Examined:*

CANADA: **Quebec:** Bonaventure Co., DAOM *3788*; Burnet, JWG *501*; Gatineau Park, DAOM *3828*F; JWG *112*; JWG *809*. **Ontario:** Timagami Forest Reserve, TRT *3530*, JWG *39*; TRT *3526*, JWG *47*; TRT *6569*, JWG *265*; JWG *302*; JWG *481*; Charleston Lake, JWG *95*; Toronto, TRT *3127*; JWG *142*; Corkery, JWG *645*.

UNITED STATES: **Massachusetts:** Princeton, JRH *1105*, JWG *347*; Huntingdon, JRH *1145*, JWG *367*; Topsfield, JRH *1513*, JWG *376*; Hamilton, JRH *1514*, JWG *377*. **Connecticut:** Hamden, JRH *1518*, JWG *379*. **Vermont:** Waterford, JRH *1102*, JWG *345*; Sharon, JRH *1520*, JWG *381*. **New York:** Lake Placid, JRH *1143*, JWG *365*; Dannemorra, JRH *1144*, JWG *366*; Canadice, JRH *1538*, JWG *388*; Alder Creek, JRH *1542*, JWG *392*. **Idaho:** Bonner Co., UIFP *2490*, JWG *761*; UIFP *19808*, JWG *781*. **California:** Mt. Shasta, WBC *18043*, JWG *833*; WBC *20487*, JWG *867*.

EUROPE: **Denmark:** Gelskov, leg. O. Rostrup, JRH.

The confusion which arose in the literature concerning *T. pithya* Karst. and *Cenangium pithyum* Fr. has been discussed in detail by Groves and Leach (1949 (10) ) and need not be recapitulated here. The two fungi are based on different types and are quite distinct.

The type of *T. pithya* Karst. is Karst. Fung. Fenn. *661* that was issued under the name *Patellaria pithya* Karst. n. sp. with a description on the label. The specimen under this number in Kew Herbarium and a slide from the specimen in the Farlow Herbarium have been examined. They agree with Karsten's description and with the fungus placed here. It is a species occurring on pine, with broadly ellipsoid to subglobose primary ascospores, and with most of the mature multispored asci measuring 15-18μ in diameter.

**4. Tympanis piceae** sp. nov. (Figs. 4, 50).

Apotheciis erumpentibus, dispersis, caespitosis vel solitariis, sessilibus vel substipitatis, versus basim attenuatis, orbicularibus vel undulatis, 0.5-1.2 mm. diam., 0.5-1.5 mm. altis, atris, duris, corneis in sicco, cartilagineis in humido; hymenio concavo vel plano, atro, subviride et carnoso in humido, marginato; hypothecio plectenchymato; ascis cylindraceis, breve stipitatis, primo tetrasporis vel octosporis, dein multisporis, (100)-115-130-(140) × (12)-14-16-(19)$\mu$; ascosporis primariis hyalinis, ellipsoideis vel subglobosis, continuis vel uniseptatis, uniseriatis, 5.0-7.5 × 3.0-4.0$\mu$; ascosporis secundariis hyalinis, unicellularis, cylindricis vel allantoideis, 2.0-3.0 × 1.0-1.5$\mu$; paraphysibus hyalinis, filiformibus, septatis, simplicibus vel ramosis, 1.5-2.0$\mu$ diam., ad apicem leviter incrassatis agglutinatisque epithecium fuscum formantibus.

Hab.: *Picea* spp.

Apothecia erumpent, scattered, cespitose or occasionally single, sessile to substipitate, narrowed below, circular to undulate, 0.5-1.2 mm. in diameter, 0.5-1.5 mm. in height, black, glabrous, hard, horny in consistency, becoming more cartilaginous when moist; hymenium concave to plane, black, becoming greenish when moist, more fleshy than the excipulum, at first with a thick, raised margin which later may disappear; tissue of the hypothecium plectenchymatous composed of interwoven, ascending, brownish to nearly hyaline hyphae about 3-5$\mu$ in diameter with the walls thickened and gelatinized, at the outside becoming thicker and darker forming a rindlike excipulum; asci cylindric, obtuse at the apex, narrowed below to a short stalk, at first with the walls thickened and gelatinized, becoming thinner with maturity, at first 4-8-spored, finally multispored, (100)-115-130-(140) × (12)-14-16-(19)$\mu$; primary ascospores hyaline, one- or two-celled, broadly ellipsoid to subglobose, uniseriate, 5.0-7.5 × 3.0-4-0$\mu$; secondary ascospores hyaline, one-celled, cylindric to allantoid, 2.0-3.0 × 1.0-1.5$\mu$; paraphyses hyaline, filiform, septate, simple or branched, 1.5-2.0$\mu$ in diameter, the tips slightly swollen and embedded in a brownish, gelatinous matrix, forming an epithecium.

Conidial fruiting bodies erumpent, cespitose, usually arising on the same stroma as the apothecia, black, glabrous, subglobose to ovoid, 0.1-0.3 mm. in diameter, similar in consistency and tissue structure to the apothecia, containing a single, subglobose to ovoid cavity; conidiophores lining the cavity, hyaline, filiform, septate, simple, not observed branching, 15-50 × 2.0-2.5$\mu$; conidia hyaline, one-celled, cylindric to allantoid, 2.0-3.0 × 1.0-1.5$\mu$, borne at the tip and along the sides of the conidiophore.

*Host:* *Picea glauca* (Moench) Voss, *Picea abies* (L.) Karst.

*Type:* DAOM *23805*, on *Picea glauca*, Bear Island, Timagami Forest Reserve, Ontario. J. W. Groves, Sept. 11, 1935. Co-type JWG *424*.

*Specimens Examined:*

EUROPE: **Sweden:** Åre. DAOM *26990*, JWG *956*.

This species is somewhat similar to *T. laricina* in gross appearance and cultural characters but differs in the larger asci and subglobose primary

ascospores. The asci are mostly slightly smaller than those of *T. pithya* and larger than those of *T. truncatula* and *T. tsugae*, the other conifer-inhabiting species with subglobose primary ascospores.

It is a rare species. After the 1935 collection, which has been designated the type, the fungus was not encountered again until 1950 when a collection made at Åre, Sweden, proved to be the same species.

5. TYMPANIS TRUNCATULA (Pers. ex Fr.) Rehm. Rab. Kr.-Fl. I, 3 : 277. 1889. (Figs. 6, 41)

*Peziza truncatula* Pers. Syn. fung. p. 673. 1801.

*Peziza truncatula* Fr. Syst. Myc. 2 : 177. 1822.

*Cycledum truncatulum* Wallr. Flor. Crypt. Germ. 2 : 512. 1833.

*Lecanidion truncatulum* Sacc. Syll. Fung. 8 : 800. 1889.

*Tympanis pinastri* sensu Tul. Sel. Fung. Carp. 3 : 139. 1865. (Not *Cenangium pinastri* Fr. Syst. Myc. 2 : 184. 1822)

Apothecia erumpent, gregarious, cespitose or occasionally single, sessile, narrowed below, sometimes somewhat turbinate, circular or undulate, 0.4-1.2 mm. in diameter, 0.4-1.0 mm. in height, usually grayish-pruinose, sometimes black and glabrous, hard, horny in consistency, becoming more cartilaginous when moist; hymenium concave to plane, grayish-pruinose or black, more fleshy than the excipulum, the margin slightly paler and usually more or less inrolled, sometimes disappearing when old; tissue of the hypothecium plectenchymatous, composed of interwoven, ascending, brownish to nearly hyaline hyphae about 2.0-4.0$\mu$ in diameter with the walls thickened and gelatinized, at the outside thicker and darker forming a rindlike excipulum; asci cylindric, obtuse at the apex, narrowed below to a short stalk, at first with the walls thickened and gelatinized, becoming thinner with maturity, at first eight-spored, finally multispored, (85)-100-130-(150) $\times$ (10)-12-15-(18)$\mu$; primary ascospores hyaline, broadly ellipsoid to subglobose, one- or two-celled, uniseriate, 5.0-7.0 $\times$ 3.0-4.0$\mu$; secondary ascospores hyaline, one-celled, cylindric to allantoid, 2.0-3.0 $\times$ 1.0-1.5$\mu$; paraphyses hyaline, filiform, septate, simple or branched, 1.5-2.0$\mu$ in diameter, the tips slightly swollen and embedded in a brownish, gelatinous matrix, forming an epithecium.

Conidial fruiting bodies usually scarce, erumpent, cespitose or single, subglobose to ovoid, 100-200$\mu$ in diameter and up to 300$\mu$ in height, black, glabrous, containing a single ellipsoid to ovoid cavity, opening at the top, consistency and tissue structure similar to the apothecia; conidiophores lining the cavity, hyaline, filiform, septate, simple, not observed branching, about 15-30 $\times$ 1.5-2.0$\mu$; conidia hyaline, one-celled, cylindric to allantoid, 2.0-3.0 $\times$ 1.0-1.5$\mu$, borne at the tip and along the sides of the conidiophore.

*Host:* *Abies* spp.

*Type:* Persoon Herb. *910.261.592*, on *Abies alba* (fide C. R. Metcalfe).

*Exsiccati:* ? Roum. Fung. Sel. Gall. Exs. *162* (as *Peziza pinastri* Pers.); Lundell & Nannf. Fung. exs. suec. *1395*, (as *Tympanis pithya*).

*Specimens Examined:*

CANADA: **Newfoundland:** Riverhead, JRH *1196*. **Nova Scotia:** Halifax, LEW *36*; Truro, LEW *1717*, JWG *526*. **Ontario:** Petawawa For. Exp. Stn., JWG *766*; Algonquin Park, JWG *885*; TRT *22338*; Timagami Forest Reserve, JWG *164*; JWG *261*; JWG *295*; JRH *2228*, DAOM *2548*; TRT *2062*; DAOM *23428*, JWG *490*.

UNITED STATES: **New Hampshire:** Carroll, JWG *386*, JRH *1526*; White Mts., FH unnumbered; Shelburne, Sept. 1891 FH unnumbered; Aug. 1894 FH unnumbered; Feb. 1889 FH unnumbered. **New York:** Lake Placid, FH unnumbered, MICH. unnumbered; Newcomb, JRH *1098*, JWG *342*; Wilmington, JRH *1139*, JWG *364*. **Michigan:** Cheboygan, JRH *1508*, JWG *372*. **Idaho:** Bonner Co., JWG *755*, UIFP *1992*H.

EUROPE: Persoon Herb. *910.261.382* (as *Peziza pinastri*); Chartreux, France, 25-7-1857, P. (*Tympanis pinastri* sensu Tul.).

In the course of this study it soon became evident that two distinct species of *Tympanis* occurred on *Abies*. They could be distinguished by the shape of the primary ascospores, the size of the asci, and the cultural characters. In the literature 10 species of *Tympanis* have been reported on *Abies* and it was necessary to determine whether any of these could be identified with one or both of these species.

*T. laricina* and *T. pithya* may be eliminated as misidentifications because these species are considered to be restricted to *Larix* and *Pinus* respectively. *T. farinacea* is also probably a misidentification for examination of the type of this species showed it to be *Cenangium ferruginosum* Fr., which occurs only on *Pinus*.

It has not been possible to see a specimen of *T. abietis* Cr. but it was described with globose secondary ascospores which would appear to exclude it from the genus *Tympanis*, and certainly eliminate it as a name for either of these species.

*T. pini* Lib. is a *nomen nudum* and examination of a specimen in Kew Herbarium revealed only an imperfect fungus that could not be identified.

The type of *T. buchsii* (P. Henn.) Rehm was examined by von Höhnel (1918*a* (18)) who stated that it was immature *Tryblidiopsis pinastri* (Fr.) Karst. This is in agreement with Rehm (1912 (31)) who could only find immature spores $18 \times 10\mu$ in the type but placed the fungus in *Tympanis* on the strength of the original description by Hennings (1903 (13)), who described the spores as $2.5\text{-}3.0 \times 0.5\mu$.

A specimen of *T. microtheca* (Karst.) Rehm was kindly loaned by Dr. D. P. Rogers from the herbarium of the New York Botanical Garden. It agreed with the original description but is not a *Tympanis*.

*T. atra* (Rab.) Karst. is an illegitimate name, based on a specimen in Rab. fung. eur. *33* labeled *Lecanidion atrum*, and apparently is a misidentification of *Patellaria atrata* Fr. According to the Rules *T. atra* must be regarded as a synonym of *P. atrata* although the fungus in Rab. fung. eur. *33* is an immature *Tympanis*.

*T. amphiboloides* was originally described by Nylander (1868 (24) ) as occurring on *Abies* and was said to have small apothecia 0.4-0.5 mm. in diameter, with asci 85 × 11-16$\mu$, and hyaline, fusiform, septate, primary ascospores 18-23 × 2.5-3.5$\mu$. No secondary ascospores were described for the species, but reference was made to them in the description of the var. *hypopodiza*. The latter has been determined to be a synonym of *T. confusa* Nyl. and is unquestionably a distinct species occurring on *Pinus*. Unfortunately no authentic material of *T. amphiboloides* has been examined, but the description agrees very closely with *Pragmopora amphibola* Massal. and, in view of Nylander's failure to find secondary ascospores, it seems very probable that *T. amphiboloides* is a *Pragmopora* and possibly a synonym of *P. amphibola*.

*T. pinastri* is a name that has been involved in much confusion. Tulasne (1865 (43) ) described a fungus said to be on *Abies* that he identified as *Cenangium pinastri* Fr. and transferred to *Tympanis*. The fungus was unquestionably a *Tympanis* and was so well described that Tulasne's account had much to do with the subsequent clear-cut concept of the genus *Tympanis*. A further result was that in the absence of critical studies of the species of *Tympanis*, collectors were inclined to call all *Tympanis* specimens found on conifers *T. pinastri*, and the name has been applied more or less indiscriminately to other species.

However, since Tulasne identified his fungus as *Cenangium pinastri* Fr. the application of his name must, according to the International Rules, be determined by Fries' type. *Cenangium pinastri* Fr. is not a *Tympanis* but is the fungus commonly known as *Tryblidiopsis pinastri* (Fr.) Karst. Therefore, *Tympanis pinastri* Tul. is an obligate synonym of *Tryblidiopsis pinastri* (Fr.) Karst. and the combination is not available for any species of *Tympanis*.

An amendment to the International Rules adopted in Stockholm, 1950, stated that in some cases it may be desirable to adopt the type of a 'prestarting-point author' and in this instance Fries had based *Cenangium pinastri* on *Peziza pinastri* Pers. During the summer of 1950 the author had the opportunity of examining the Persoon collections in the Rijksherbarium, Leiden, and five specimens were found labeled *Peziza pinastri* and bearing the numbers *910.261.381*, *910.261.382*, *910.261.384*, *910.261.388*, and *910.261.390*.

No. *381* bears only the name *Peziza pinastri* in Persoon's handwriting and consists of excellent material of a *Tympanis*, apparently the species described in this paper as *T. abietina*.

The label of No. *382* is not in Persoon's handwriting. It is apparently a specimen that had been sent to him by Chaillet. It is the same as Tulasne's species and is in good condition.

The label of No. *384* is in Persoon's handwriting with "Moug. in herb. Pers." written in a different hand. This proved to be a *Tympanis* of the *hypopodia* group and seems to be the same as the fungus described in this paper as *T. piceina*, but the host was not stated. It is not in very good condition.

The label of No. *388* is not in Persoon's handwriting and bears a ? after the name. The fungus is *Cenangium ferruginosum* Fr.

The label of No. *390* is in Persoon's handwriting but also bears a ? and the fungus is an immature and unidentifiable *Tympanis*.

Thus Nos. *388* and *390* are quite unsuitable as types and No. *384* is unsatisfactory because of the poor condition and uncertainty as to the exact identity of the fungus. It would be difficult to justify the choice of No. *382* since it is evidently a specimen identified by someone else, presumably Chaillet, and sent to Persoon, and it is a different species from the specimen in Persoon's own handwriting. This leaves only No. *381* as a possible type and its choice would not only fail to retain the name in Tulasne's sense but would also upset the nomenclature of the fungus now known as *Tryblidiopsis pinastri*, which would require a new name.

Presumably the amendment referred to above was worded in this way in order to permit the adoption of the type of a prestarting-point author where this would contribute to greater stability of nomenclature, but to invoke this rule in this instance would obviously upset the nomenclature. Furthermore, the name *Tympanis pinastri* has been used in a collective sense to include a number of different species and is thus a source of confusion. It seemed undesirable to recognize a Persoon specimen as type merely to preserve the specific epithet "pinastri" for a species of *Tympanis*, especially when it would be a different species from the one described by Tulasne. The treatment of *T. pinastri* by Seaver (1951 (38) ) is untenable from any viewpoint.

*Peziza truncatula* was described by Persoon (1801 (26) ) and said to be "*ad corticem fagorum*". Later (1822 (27) ), he again described *P. truncatula* and in this account gave the host as "*ad truncos Fagi* ?", indicating that he had some doubt concerning the identification of the host.

In the Persoon Herbarium at Leiden there is a specimen No. *910.261.592* labeled *Peziza truncatula* Pers. which must be the type, and this is a *Tympanis* in excellent condition. Through the kindness of Dr. R. A. Maas Geesteranus, a fragment of bark from this specimen was sent to Dr. C. R. Metcalfe, Royal Botanic Gardens, Kew, who identified it as *Abies alba*.

The asci in this specimen measured 90-135 × 11-14$\mu$ and the primary ascospores were broadly ellipsoid to subglobose in the mount examined. It is undoubtedly the same fungus as that described by Tulasne (1865 (43) ) as *T. pinastri*.

Rehm (1889 (30) ), relying solely on Persoon's inadequate description, referred a *Tympanis* collected on *Fagus* by von Tubeuf to *Peziza truncatula* and made the combination *Tympanis truncatula* although he was very doubtful of the identification. However, according to the International Rules, Rehm's combination must be considered to be based on Persoon's type and is an obligate synonym of *Peziza truncatula* Pers. This is, therefore, the correct name for this species occurring on *Abies*. Since no name appears to be available for the other species on *Abies*, it is described as a new species, *Tympanis abietina*.

The apothecia of *T. truncatula* are frequently grayish-pruinose and usually more turbinate in shape than those of *T. abietina*. However, they cannot be

distinguished with certainty by the gross appearance but can readily be distinguished microscopically. The primary ascospores of *T. truncatula* are ovoid to subglobose whereas those of *T. abietina* are subfusiform. The mature asci of *T. truncatula* mostly exceed 100$\mu$ in length and 12$\mu$ in width, whereas those of *T. abietina* rarely exceed these dimensions.

The two species can be readily separated also on cultural characters. Cultures of *T. truncatula* on malt extract agar produce very little aerial mycelium with few pycnidia, and the margin of the colony is indistinct. Cultures of *T. abietina* produce considerable brownish, aerial mycelium with abundant pycnidia, and the margin of the colony is abrupt and clear-cut. They also differ in growth rate, colonies of *T. truncatula* reaching about 9 cm. in three weeks, and colonies of *T. abietina* reaching about 2.5-3.0 cm. in the same time.

The material distributed in Roum. Fung. Sel. Gall. Exs. *162* is probably a mixture of species for the label gives the host as *Pinus*, *Picea*, and *Abies*. The specimen in the herbarium of the Division of Botany and Plant Pathology, Ottawa, is apparently all *T. truncatula*.

6. **Tympanis tsugae** sp. nov. (Figs. 7, 49)

Apotheciis erumpentibus, gregariis, caespitosis, raro solitariis, sessilibus, versus basim leviter attenuatis, orbicularibus vel undulatis, 0.5-0.8 mm. diam., 0.3-0.6 mm. altis, atris, glabris vel griseo-pruinosis, duris, corneis in sicco, cartilagineis in humido; hymenio concavo vel plano, atro, carnoso in humido, marginato; hypothecio plectenchymato; ascis cylindraceis, breve stipitatis, primo octosporis, dein multisporis, (70)-90-125-(140) × (9)-11-14-(17)$\mu$; ascosporis primariis, hyalinis, ellipsoideis vel subglobosis, continuis vel uniseptatis, uniseriatis, 5-7 × 3-4$\mu$; ascosporis secundariis hyalinis, cylindricis vel allantoideis, unicellularis, 2.0-4.0 × 1.0-1.5$\mu$; paraphysibus hyalinis, filiformibus, septatis, simplicibus vel ramosis, 1.5-2.0$\mu$ diam., ad apicem leviter incrassatis agglutinatisque, epithecium fuscum formantibus.

Hab.: *Tsuga canadensis* (L.) Carr.

Apothecia erumpent, gregarious, cespitose or occasionally single, sessile, slightly narrowed below, circular or undulate, 0.5-0.8 mm. in diameter, 0.3-0.6 mm. in height, black, glabrous or sometimes grayish-pruinose, hard, horny in consistency, becoming more cartilaginous when moist; hymenium concave to plane, black, more fleshy than the excipulum, with a rather persistent, raised, and slightly inrolled margin; tissue of the hypothecium plectenchymatous composed of interwoven, ascending, brownish to nearly hyaline hyphae about 2.0-5.0$\mu$ in diameter with the walls thickened and gelatinized, at the outside becoming thicker and darker forming a rindlike excipulum; asci cylindric, obtuse at the apex, narrowed below to a short stalk, at first with the walls thickened and gelatinized, becoming thinner with maturity, at first eight-spored, finally multispored, (70)-90-125-(140) × (9)-11-14-(17)$\mu$; primary ascospores hyaline, ellipsoid to subglobose, one- or two-celled, uniseriate, 5-7 × 3-4$\mu$; secondary ascospores hyaline, cylindric to allantoid, one-celled, 2.0-4.0 × 1.0-1.5$\mu$; paraphyses hyaline, filiform,

septate, simple or branched, 1.5-2.0$\mu$ in diameter, the tips swollen slightly and embedded in a brownish, gelatinous matrix, forming an epithecium.

Conidial fruiting bodies erumpent, cespitose or occasionally single, usually arising from the same stroma as the apothecia, black, glabrous, subglobose to ovoid, 120-300$\mu$ in diameter, 200-400$\mu$ in height, consistency and tissue structure similar to the apothecia, containing a single, subglobose to ovoid cavity, opening at the top; conidiophores lining the cavity, hyaline, filiform, septate, simple or branched, 10-40 $\times$ 1.5-2.0$\mu$; conidia hyaline, cylindric to allantoid, one-celled, 2.0-4.0 $\times$ 1.0-1.5$\mu$, borne at the tip and along the sides of the conidiophore.

*Host:* *Tsuga canadensis* (L.) Carr.

*Type:* TRT *7208*, Don Valley, Toronto, Ontario, Coll. J. W. Groves, Oct. 28, 1933. Co-type, JWG *140*, DAOM *23808*.

*Specimens Examined:*

CANADA: **Quebec:** Burnet, JWG *823*; Chelsea, JWG *510*; Kingsmere, JWG *114*.

UNITED STATES: **Maine:** Indian Town, JRH *2262*. **Vermont:** Brandon, JRH *2263*. **Connecticut:** N. Bloomfield, JRH *1523*, JWG *384*. **New York:** E. Galway, FH.

This species is characterized by the rather small, not strongly erumpent apothecia, rather long, slender asci, and ellipsoid primary ascospores. Four species with ellipsoid primary ascospores are recognized as occurring on conifers. Of these, *T. pithya* and *T. piceae* both have larger asci and, although it is fairly close to *T. truncatula* microscopically, the latter has larger, more strongly erumpent apothecia. *T. tsugae* is also distinct from all three culturally. The cultures are slow-growing, whitish, and produce abundant conidial fruiting bodies. Apothecia have been produced several times in twig cultures.

7. TYMPANIS LARICINA (Fckl.) Sacc., Syll. Fung. 8 : 583. 1889. (Figs. 2, 43)
*Cenangium laricinum* Fckl., Symb. Myc. p. 270. 1870.

Apothecia erumpent, scattered, separate or cespitose in small, usually more or less elongated clusters, sessile, narrowed below to substipitate, circular or undulate, 0.5-1.0 mm. in diameter, 0.4-1.3 mm. in height, black, glabrous, hard, horny in consistency, becoming more cartilaginous when moist; hymenium at first concave, becoming plane to convex, black or greenish when moist, more fleshy than the excipulum, at first with a thick, raised margin which later may almost disappear; tissue of the hypothecium plectenchymatous, composed of interwoven, ascending, brownish to nearly hyaline hyphae about 3-5$\mu$ in diameter, with the walls thickened and gelatinized, at the outside becoming thicker and darker forming a rindlike excipulum; asci cylindric, obtuse at the apex, narrowed below to a short stalk, at first with the walls thickened and gelatinized, becoming thinner with maturity, at first eight-spored, finally multispored, (70)-80-110-(120) $\times$ (11)-13-15-(17)$\mu$; primary ascospores hyaline, ellipsoid–fusiform, one- or two-celled, irregularly

biseriate to uniseriate, 7-10 × 3-4μ; secondary ascospores hyaline, one-celled, cylindric to allantoid, 2.0-3.0 × 1.0-1.5μ; paraphyses hyaline, filiform, septate, simple or branched, about 1.5-2.5μ in diameter, the tips slightly swollen and embedded in a brownish, gelatinous matrix, forming an epithecium.

Conidial fruiting bodies erumpent, cespitose, in clusters of up to 12-15, occasionally single, the clusters 0.5-1.0 mm. in diameter and 0.4-1.0 mm. in height, the pycnidia subglobose to ovoid, black, glabrous, 0.2-0.3 mm. in diameter, consistency and tissue structure similar to the apothecia, containing a single globose to ovoid cavity, opening at the top; conidiophores lining the cavity, hyaline, filiform, septate, not observed branching, about 15-50 × 1.5-2.0μ; conidia hyaline, one-celled, cylindric to allantoid, 2.0-3.0 × 1.0-1.5μ, borne at the tip and along the sides of the conidiophore.

*Host:* *Larix* spp.

*Type:* Fuckel Fung. Rhen. *2473* (as *Cenangium laricinum*).

*Exsiccati:* Phill. Elv. Brit. *145* (as *Cenangium laricinum*); ? Erb. critt. ital. *822* (as *C. laricinum*).

*Specimens Examined:*

CANADA: **Ontario:** Timagami Forest Reserve, TRT *8013*, JWG *427*; TRT *8014*, JWG *322*; Algonquin Park, TRT *15943*, DAOM *14856*.

UNITED STATES: **New Hampshire:** Chocorua, FH. **Massachusetts:** Ipswich, JRH *2261*. **Idaho:** Bonner Co., UIFP *1693*B, DAOM *12057*, JWG *749*; UIFP *1942*B, DAOM *12055*, JWG *754*; Shoshone Co., UIFP *2981*, JWG *747*.

EUROPE: **Austria:** Nassau, FH ex herb. Barbey-Boissier *1103*; FH ex herb. Barbey-Boissier *1106*; FH ex herb. Barbey-Boissier *1107*.

*Tympanis laricina* was originally described by Fuckel (1870 (7) ) as *Cenangium laricinum*. He described both a perfect and an imperfect state but it is improbable that these two fungi were genetically related. He described the conidia as long, filiform, and curved, and the description suggests a species of *Micropera*. Cultural studies of the *Tympanis* on *Larix* have given no evidence of the connection of such a conidial state but have proved the connection of a typical *Pleurophomella* such as is connected with other species of *Tympanis*.

No specimens were cited in the original description but later Fuckel (1873 (8) ) stated that the apothecial state had been distributed in Fung. Rhen. *2473* which, presumably, should be considered the type. The specimens under this number in the Farlow Herbarium, Kew Herbarium, and British Museum have been examined and agree with the North American material. The specimen of Erb. critt. ital. *822* in the British Museum, was quite immature but certainly a *Tympanis*.

A specimen was issued in Erb. critt. ital. *1284* under the name *Tympanis laricina* Pass. with a reference to *Cenangium laricinum* Fckl. as a questionable synonym. This fungus is not a *Tympanis* but appears to be the fungus recently described by Ettlinger (1945 (4) ) as *Crumenula laricina*. *Tympanis*

*laricina* Pass. is a *nomen nudum* but Saccardo (1889 (34) ) supplied a description and transferred it to *Cenangium*. At the same time he transferred *Cenangium laricinum* Fckl. to *Tympanis* where it properly belongs, but this gave rise to confusion in the nomenclature.

Obviously *Cenangium laricinum* (Pass.) Sacc. is illegitimate since it is a later homonym of *C. laricinum* Fckl. and, therefore, the correct name for this fungus is at present *Crumenula laricina* Ettl. although it is not congeneric with the type of *Crumenula*.

The International Rules state that a *nomen nudum* does not invalidate later use of the name, and also that a name must be accepted by its author before it is validly published. Therefore, for nomenclatural purposes *Tympanis laricina* Pass. may be ignored. The question mark with reference to *Cenangium laricinum* Fckl. invalidates the combination on the basis of its being a *nomen provisorium*, and since it is a *nomen nudum* it does not invalidate Saccardo's later use of the name. *Tympanis laricina* (Fckl.) Sacc. can thus be accepted as the valid name of this species.

A specimen of Rehm Ascom *773* in the Farlow Herbarium labeled *Tympanis pinastri* f. *laricinum* was examined. It was immature but was certainly a *Tympanis* and probably this species.

All of the specimens examined occurring on *Larix*, whether European or North American, appear to belong to this species although no European material has been studied in culture. By reason of the ellipsoid–fusiform ascospores and small asci, it shows a close relationship to the "hypopodia" group, but the asci become both longer and broader than in other species of this group.

8. TYMPANIS HYPOPODIA Nyl. Obs. Pez. Fenn. p. 72. 1868. (Figs. 5, 46)

Apothecia erumpent, gregarious, separate to cespitose with usually less than six in a cluster, circular or undulate, sessile to substipitate, narrowed below, 0.5-1.0 mm. in diameter, 0.3-1.5 mm. in height, black, glabrous, hard, horny in consistency, becoming more cartilaginous-fleshy when moist; hymenium concave to plane, black, more fleshy than the excipulum, at first with a thick, somewhat infolded margin which later may disappear; tissue of the hypothecium plectenchymatous, composed of interwoven, ascending, brownish to nearly hyaline hyphae about 1-2$\mu$ in diameter, with the walls greatly thickened and gelatinized, becoming thicker and darker at the outside, forming a rindlike excipulum, subhymenium not clearly differentiated; asci cylindric to cylindric-clavate, short-stalked, at first with the walls thickened and gelatinized becoming thinner with maturity, at first eight-spored, finally multispored, (60)-70-90-(110) $\times$ (8.5)-9.0-12.0-(14.0)$\mu$; primary ascospores hyaline, fusiform, one- or two-celled, irregularly biseriate, (6)-8-10-(12) $\times$ 2.0-4.0$\mu$; secondary ascospores hyaline, cylindric to allantoid, one-celled, 2.0-3.0 $\times$ 1.0-1.5$\mu$; paraphyses hyaline, filiform, septate, simple or branched, 1.5-2.0$\mu$ in diameter, the tips slightly swollen up to 3.0$\mu$ and embedded in a gelatinous matrix forming a brownish epithecium.

Conidial fruiting bodies erumpent, gregarious to scattered, separate or cespitose, black, glabrous, globose to ovoid or cylindric, sessile, 0.1-0.3 mm. in diameter, 0.1-0.3 mm. in height, similar in consistency to the apothecia; tissue plectenchymatous, similar to that of the apothecia, in the upper part containing a globose to ovoid cavity lined with conidiophores and tearing open at the top; conidiophores hyaline, filiform, septate, simple, 25-70 × 2.0-2.5$\mu$; conidia hyaline, cylindric to allantoid, one-celled, borne at the tip and along the sides of the conidiophore, 2.0-4.0 × 1.0-1.5$\mu$.

*Host:* *Pinus* spp.

*Exsiccati:* Karst. Fung. Fenn. *754*; Rel. Farl. *156B* (as *T. pinastri*) Rab. Fung. Eur. *1231* (as *T. pinastri*).

*Specimens Examined:*

CANADA: **Ontario:** Timagami Forest Reserve, JWG *327*; JWG *300*; Petawawa Forest Experiment Station, JWG *564*; Corkery, JWG *879*.

UNITED STATES: **Massachusetts:** Sunderland, JRH *6*, JWG *334*; Ipswich, JRH *1512*, JWG *375*. **Connecticut:** East Granby, JRH *1104*, JWG *346*. **Idaho:** Shoshone Co., UIFP *2343*, JWG *707*; Bonner Co., UIFP *2726*, JWG *763*; UIFP *1848A*, JWG *780*; Clearwater Natl. Park, DAOM ex herb. J. R. Weir, *16615*.

EUROPE: **Sweden,** UPS.

*T. hypopodia* was discussed in detail by Groves and Leach (1949 (10) ). The type has not been seen. It was collected by Karsten and described by Nylander (1868 (24) ). A later collection made by Karsten was distributed in Karst. Fung. Fenn. *754* and this has been examined and agrees with the original description. It has been confused with *T. pithya* but can be readily distinguished by the much smaller asci and fusiform primary ascospores.

A group of species morphologically similar to *T. hypopodia* occurs on various conifers. Some might prefer to regard all of these as varieties of one species and, in that case, *T. hypopodia* is the oldest name. However they seem to be culturally distinct and I prefer to regard them as species at present.

9. **Tympanis abietina** sp. nov. (Figs. 9, 42)

Apotheciis, erumpentibus, gregariis, solitariis vel caespitosis, sessilibus, versus basim attenuatis, orbicularibus vel undulatis, 0.5-1.2 mm. diam., 0.4-1.0 mm. altis, atris, duris, corneis in sicco, cartilagineis in humido; hymenio concavo vel plano, atro, subviride et carnoso in humido; hypothecio plectenchymato; ascis cylindraceis, breve stipitatis, primo octosporis dein multisporis, (65)-75-95-(110) × (8.5)-9.5-12-(14)$\mu$; ascosporis primariis hyalinis, fusiformibus, continuis vel uniseptatis, irregulariter biseriatis, 7-10 × 2.0-3.0$\mu$; ascosporis secundariis hyalinis, unicellularis, cylindraceis vel allantoideis, 2.0-3.0 × 1.0-1.5$\mu$; paraphysibus hyalinis, filiformibus, septatis, simplicibus vel ramosis, 1.5-2.0$\mu$ diam. ad apicem leviter incrassatis agglutinatisque, epithecium fuscum formantibus.

Hab.: *Abies* spp.

Apothecia erumpent, usually densely gregarious, separate or cespitose in small clusters, sessile, narrowed below, circular or undulate, 0.5-1.2 mm. in diam., 0.4-1.0 mm. in height, black, glabrous, hard, horny in consistency, becoming more cartilaginous when moist; hymenium concave to plane, black or greenish when moist, more fleshy than the excipulum, at first with a thick, raised or somewhat inrolled margin which later may disappear; tissue of the hypothecium plectenchymatous, composed of interwoven, ascending, brownish to nearly hyaline hyphae about 2-3$\mu$ in diam. with the walls thickened and gelatinized, at the outside thicker and darker forming a rindlike excipulum; asci cylindric, obtuse at the apex, narrowed below to a short stalk, at first with the walls thickened and gelatinized, becoming thinner with maturity, at first eight-spored, finally multispored, (65)-75-95-(110) $\times$ (8.5)-9.5-12-(14)$\mu$; primary ascospores hyaline, fusiform, one- or two-celled, irregularly biseriate, 7-10 $\times$ 2.0-3.0$\mu$; secondary ascospores hyaline, one-celled, cylindric to allantoid, 2.0-3.0 $\times$ 1.0-1.5$\mu$; paraphyses hyaline, filiform, septate, simple or branched, 1.5-2.0$\mu$ in diameter, the tips slightly swollen and embedded in a brownish, gelatinous matrix forming an epithecium.

Conidial fruiting bodies erumpent, scattered, separate or occasionally cespitose, subglobose to ovoid or conical, black, glabrous, 0.2-0.3 mm. in diameter, 0.2-0.4 mm. in height, consistency and tissue structure similar to the apothecia, containing a single globose to ovoid cavity, opening at the top; conidiophores lining the cavity, hyaline, filiform, septate, simple or branched, about 15-50 $\times$ 1.5-2.0$\mu$; conidia hyaline, one-celled, cylindric to allantoid, 2.0-4.0 $\times$ 1.0-1.5$\mu$, borne at the tip and along the sides of the conidiophore.

*Host:* *Abies* spp.

*Type:* DAOM *14857*. On *Abies balsamea* (L.) Mill., Timagami Forest Reserve, Ontario. Sept. 3, 1934. Co-type JWG *256*, TRT *6774*.

*Specimens Examined:*

CANADA: **Newfoundland:** Sand Lake, JRH *1197*; Virginia Water, FH. **Nova Scotia:** LEW *30* in Herb. Univ. Mich.; Colch. Co., LEW *1658*, JWG 527. **New Brunswick:** Campobello, FH. **Quebec:** Duchesnay, DAOM *5306*, JWG *620*. **Ontario:** South March, JWG *504*; Petawawa For. Exp. Stn., DAOM *4684*, JWG *563*; DAOM *7990*, JWG *765*; Timagami For. Reserve, JWG *35*; JWG *167*; JWG *191*, JRH *1140*, TRT *6968*; JWG *301*; JRH *2227*; JRH *2229*. **British Columbia:** Revelstoke, JRH *2230*.

UNITED STATES: **Maine:** Machias, JRH *1099*; Mt. Moaselanke, FH. **New Hampshire:** Chocorua, FH; Mt. Washington, JRH *1199*. **New York:** Bethlehem, JRH *1100*, JWG *343*. **Idaho:** Bonner Co., UIFP 2044B, JWG *758*. **Wyoming:** Silver Lake, JWG *953*.

EUROPE: Persoon Herb. *910.261.381* (as *Peziza pinastri*).

Of the two species occurring on *Abies*, this one appears to be the more common, at least in Ontario where most of the collections have been made. This species can be recognized by the more or less fusiform primary ascospores and by the small asci which rarely exceed 100$\mu$ in length and 12$\mu$ in diameter.

It is very close to *T. hypopodia* in morphology but seems to be constantly distinct in cultural characters.

The cultures are quite distinct from other conifer-inhabiting species, forming a brownish, velvety, slow-growing colony with an abrupt margin. Conidial fruiting bodies are produced abundantly both on agar and on sterilized twigs, and apothecia have been produced frequently in twig cultures.

The nomenclatural problems of the species occurring on *Abies* have been discussed under *T. truncatula*. The only specimen of *Peziza pinastri* Pers. in the Persoon herbarium that could reasonably be adopted as the type, is apparently this fungus, but to invoke the rule permitting the adoption of the type of a prestarting-point author would in this instance cause further confusion. It has, therefore, been decided to ignore the Persoon material as prestarting-point and describe this fungus as a new species.

10. **Tympanis piceina** sp. nov. (Figs. 8, 51)

Apotheciis erumpentibus, gregariis, solitariis vel caespitosis, sessilibus, versus basim attenuatis, orbicularibus vel undulatis, 0.4-1.0 mm. diam., 0.3-0.8 mm. altis, atris, duris, corneis in sicco, cartilagineis in humido; hymenio concavo vel plano, atro, carnoso in humido, marginato; hypothecio plectenchymato; ascis cylindraceis, breve stipitatis, primo octosporis, dein multisporis, (65)-75-95-(105) × (10)-11-13-(14)$\mu$; ascosporis primariis, hyalinis, fusiformibus, rectis vel leviter curvulis, continuis vel uniseptatis, uniseriatis vel irregulariter biseriatis, 5.0-8.0 × 2.5-3.0$\mu$; ascosporis secundariis hyalinis, unicellularis, cylindricis vel allantoideis, 2.0-3.0 × 1.0-1.5$\mu$; paraphysibus hyalinis, filiformibus, septatis, simplicibus vel ramosis, 1.5-2.0$\mu$ diam., ad apicem leviter incrassatis agglutinatisque, epithecium fuscum formantibus.

Hab.: *Picea* spp.

Apothecia erumpent, gregarious, mostly separate or occasionally cespitose, sessile, narrowed below, circular or undulate, 0.4-1.0 mm. in diameter, 0.3-0.8 mm. in height, black, glabrous, hard, horny in consistency, becoming more cartilaginous when moist; hymenium concave to plane, black, more fleshy than the excipulum, at first with a raised, somewhat inrolled margin which later may almost disappear; tissue of the hypothecium plectenchymatous composed of interwoven, ascending, brownish to nearly hyaline hyphae about 3-5$\mu$ in diameter, with the walls thickened and gelatinized, at the outside becoming thicker and darker forming a rindlike excipulum; asci cylindric, obtuse at the apex, narrowed below to a short stalk, at first with the walls thickened and gelatinized, becoming thinner with maturity, at first eight-spored, finally multispored, (65)-70-95-(105) × (10)-11-13-(14)$\mu$; primary ascospores hyaline, fusiform, one- to two-celled, straight or slightly curved, uniseriate or irregularly biseriate, 5.0-8.0 × 2.5-3.0$\mu$; secondary ascospores hyaline, one-celled, cylindric to allantoid, 2.0-3.0 × 1.0-1.5$\mu$; paraphyses hyaline, filiform, septate, simple or branched, 1.5-2.0$\mu$ in diameter, the tips slightly swollen and embedded in a brownish, gelatinous matrix, forming an epithecium.

Conidial fruiting bodies erumpent, scattered, separate or two or three arising from the same stroma, subglobose to ovoid, about 0.1-0.2 mm. in diameter, black, glabrous, similar in consistency and tissue structure to the apothecia, containing a single ovoid to subglobose cavity, opening at the top; conidiophores lining the cavity, hyaline, filiform, septate, simple, not observed branching, 20-50 × 1.5-2.0$\mu$; conidia hyaline, one-celled, cylindric to allantoid, 2.0-3.0 × 1.0-1.5$\mu$, borne at the tip and along the sides of the conidiophores.

*Host:* *Picea* spp.

*Type:* DAOM *26989*, on *Picea glauca* (Moench) Voss, St. Aubert, L'Islet Co., Que. J. W. Groves, Sept. 27, 1946. Co-type JWG *838*.

*Specimens Examined:*

UNITED STATES: **New Hampshire:** Carroll, JRH *2258*; Waterville, JRH *1114*, JWG *352*. **Vermont:** Bethel, JRH *1537*, JWG *387*; JRH *1113*. **New York:** Newcomb, JRH *2257*.

EUROPE: Persoon Herb. *910.261.384.* (as *Peziza pinastri*). **Sweden:** Handöl, DAOM *26991*, JWG *957*; K (as *Cenangium abietis* on *Abies excelsa*, coll. Keissler).

This species is one of the "hypopodia" group and is recognized as a distinct species chiefly on the basis of its occurrence on *Picea* and the fact that cultures seemed to be different from other species of this group on different hosts. It would seem to occur more frequently than *T. piceae*, for several specimens were found among the Hansbrough collections, but only the type and the specimen collected at Handöl, Sweden, have been studied in the fresh condition and cultured. It was remarkably good fortune that, of the two specimens of *Tympanis* collected on *Picea abies* in Sweden on the field trip after the International Botanical Congress, 1950, one should prove to be *T. piceae* and the other *T. piceina*. They can readily be distinguished by the size of the asci and the shape of the primary ascospores.

11. **Tympanis hansbroughiana** sp. nov. (Figs. 3, 45)

Apotheciis erumpentibus, dispersis vel gregariis, solitariis vel subcaespitosis, orbicularibus vel undulatis, sessilibus, versus basim attenuatis, atris, 0.5-1.0 mm. diam., 0.2-0.4 mm. altis, duris, corneis in sicco, cartilagineis in humido; hymenio primo concavo, dein plano vel convexo, atro, carnoso in humido; hypothecio plectenchymato; ascis cylindraceis, breve stipitatis, primo octosporis, dein multisporis, (65)-75-95-(105) × 11-13-(14)$\mu$; ascosporis primariis hyalinis, clavato-fusiformibus, continuis vel uniseptatis, uniseriatis vel sub-biseriatis, 7.0-10 × 2.5-3.0$\mu$; ascosporis secundariis hyalinis, unicellularis, cylindricis vel allantoideis, 2.0-3.0 × 1.0-1.5$\mu$; paraphysibus hyalinis, filiformibus, septatis, simplicibus vel ramosis, 2.0-2.5$\mu$ diam., ad apicem leviter incrassatis agglutinatisque, epithecium fuscum formantibus.

Hab.: *Pseudotsuga taxifolia* (Poir.) Britton.

Apothecia erumpent, scattered to gregarious, separate or in small clusters, circular or undulate, sessile, narrowed below, black, glabrous, 0.5-1.0 mm. in

diameter, 0.2-0.4 mm. in height, hard, horny in consistency, becoming more cartilaginous when moist; hymenium at first concave, becoming plane to slightly convex, black, more fleshy than the excipulum, at first with a slightly raised margin which later may disappear; tissue of the hypothecium plectenchymatous, composed of interwoven, ascending, brownish to nearly hyaline hyphae about 2.0-4.0$\mu$ in diameter with the walls thickened and gelatinized, at the outside becoming thicker and darker forming a rindlike excipulum; asci cylindric, obtuse at the apex, narrowed below to a short stalk, at first with the walls thickened and gelatinized becoming thinner with maturity, at first eight-spored, finally multispored, (65)-75-95-(105) × 11-13-(14)$\mu$; primary ascospores hyaline, clavate-fusiform, one- or two-celled, uniseriate to subbiseriate, 7.0-10.0 × 2.5-3.0$\mu$; secondary ascospores hyaline, one-celled, cylindric to allantoid, 2.0-3.0 × 1.0-1.5$\mu$; paraphyses hyaline, filiform, septate, simple or branched, about 2.0-2.5$\mu$ in diameter, the tips slightly swollen and embedded in a brownish, gelatinous matrix, forming an epithecium.

*Host:* *Pseudotsuga taxifolia* (Poir.) Britton.

*Type:* DAOM *23430*, on *Pseudotsuga taxifolia*, Harvard Forest, Petersham, Mass. Coll. J. R. Hansbrough, May 14, 1935. Co-type JWG *385*, JRH *1524*.

*Specimens Examined:*

UNITED STATES: **Oregon:** Rhododendron, JWG *354*, JRH *1116*. **Massachusetts:** Hamilton, JRH *1515*.

Only three rather scanty specimens of this species have been seen and two of these are from eastern plantations. It was rather puzzling to find an apparently new species on a host so far outside its normal range and it seemed more likely that one of the species on a native host was growing on the *Pseudotsuga*. However, cultures were obtained from JWG *385* and they did not agree with any known species occurring on the eastern conifers. Furthermore, the primary ascospores seemed to be more variable in shape in this form than in other species of the "hypopodia" group. They are somewhat clavate, sometimes ellipsoid to nearly ovoid, in contrast to the more constantly fusiform shape of the other species. One specimen from Oregon, also from Dr. Hansbrough's collections, appeared to agree morphologically with the eastern collections although it was not possible to obtain cultures from this specimen. The species is named in honor of Dr. J. R. Hansbrough who has collected all the material so far observed.

The conidial state was not observed in any of the specimens examined but ascospore cultures produced a typical *Pleurophomella* in culture.

12. TYMPANIS JUNIPERINA (Sacc.) Sacc. Syll. Fung. 8 : 584. 1889. (Fig. 44)
*Cenangium juniperinum* Sacc. Michelia 1 : 419. 1878.

Apothecia erumpent, scattered, mostly separate to subcespitose with two or three in a cluster, sessile, slightly narrowed below, circular to undulate, 0.5-0.8 mm. in diameter, 0.2-0.4 mm. in height, black, glabrous, hard, horny in consistency, becoming more cartilaginous when moist, hymenium concave to plane, black or slightly grayish-pruinose, more fleshy than the excipulum,

with a thick, slightly raised margin; tissue of the hypothecium plectenchymatous composed of brownish to nearly hyaline, ascending, interwoven hyphae about 1.5-2.5$\mu$ in diam. with the walls thickened and gelatinized, at the outside becoming thicker and darker, forming a rindlike excipulum; subhymenium not clearly differentiated; asci cylindric, narrowed below to a short stalk, at first with the wall thickened and gelatinized, becoming thinner with maturity, at first eight-spored, finally multispored 70-90-(100) × 9.5-11.0$\mu$; primary ascospores hyaline, fusiform, one- or two-celled, about 5-7 × 2-3$\mu$, uniseriate to irregularly biseriate; secondary ascospores hyaline, one-celled, cylindric to allantoid, 2.0-3.0 × 1.0-1.5$\mu$; paraphyses hyaline, filiform, septate, simple or occasionally branched, 1.5-2.0$\mu$ in diam., the tips slightly swollen and embedded in a brownish, gelatinous matrix, forming an epithecium.

*Host:* *Juniperus communis* L.

*Type:* On *Juniperus communis*. Aug. 1878. Spegazzini 1879. (Kew Herb.).

The above specimen at Kew is in a type folder and is the only specimen on *Juniperus* that I have seen. The material is fairly abundant and in good condition. No conidial fruiting bodies were found. This is the only species occurring on a conifer that has not been studied in fresh condition. It is closely related to *T. hypopodia*, but the asci are slightly smaller than the other species of this group.

13. TYMPANIS SALIGNA Tode, Fung. Meckl. 1 : 24. 1790. (Figs. 12, 52)

Apothecia immersed to slightly erumpent, separate or subcespitose with two or three in a cluster, sessile, scarcely narrowed below, circular or undulate, 0.5-0.8-(1.0) mm. in diameter, 0.2-0.3 mm. in height, densely grayish-pruinose, blackish when moist, hard, horny in consistency, becoming more cartilaginous when moist; hymenium concave, black to grayish-pruinose, more fleshy than the excipulum, with a thick, persistently inrolled margin; tissue of the hypothecium plectenchymatous, composed of interwoven, ascending, brownish to nearly hyaline hyphae about 1.5-2.5$\mu$ in diameter with the walls thickened and gelatinized, at the outside becoming thicker and darker forming a rindlike excipulum; asci cylindric, obtuse at the apex, narrowed below to a short stalk, at first with the walls thickened and gelatinized, becoming thinner with maturity, at first eight-spored, finally multispored, (135)-160-210-(230) × (15)-18-23-(25)$\mu$; primary ascospores hyaline, one-celled, broadly ellipsoid to subglobose, uniseriate, 5-8 × 4-7$\mu$; secondary ascospores hyaline, one-celled, cylindric to allantoid, 2.0-3.0 × 1.0-1.5$\mu$; paraphyses hyaline, filiform, septate, simple or occasionally branched, 1.5-2.0$\mu$ in diameter, the tips slightly swollen and embedded in a brownish, gelatinous matrix, forming an epithecium.

*Host:* *Salix* spp.

*Neotype:* Jaap Fung. Sel. Exs. *414*.

*Exsiccati:* Krieg. Fung. Sax. *2230*; Roum. Fung. Sel. Gall. Exs. *2274* (as *Peziza fascicularis*).

*Specimens Examined:*

CANADA: **Ontario:** Ottawa, DAOM *26179*, JWG *952*.

UNITED STATES: **West Virginia:** Morgantown, FH.

EUROPE: **Sweden:** Fung. Suec. *202*, UPS. **Germany:** S, 9/1888, Rehm; S, 8/1903, Rehm; S, 5/1904, Rehm; S, 5/1906, Rehm; S, 15-3-1910, Jaap; S, Aug. 1912, Krieger.

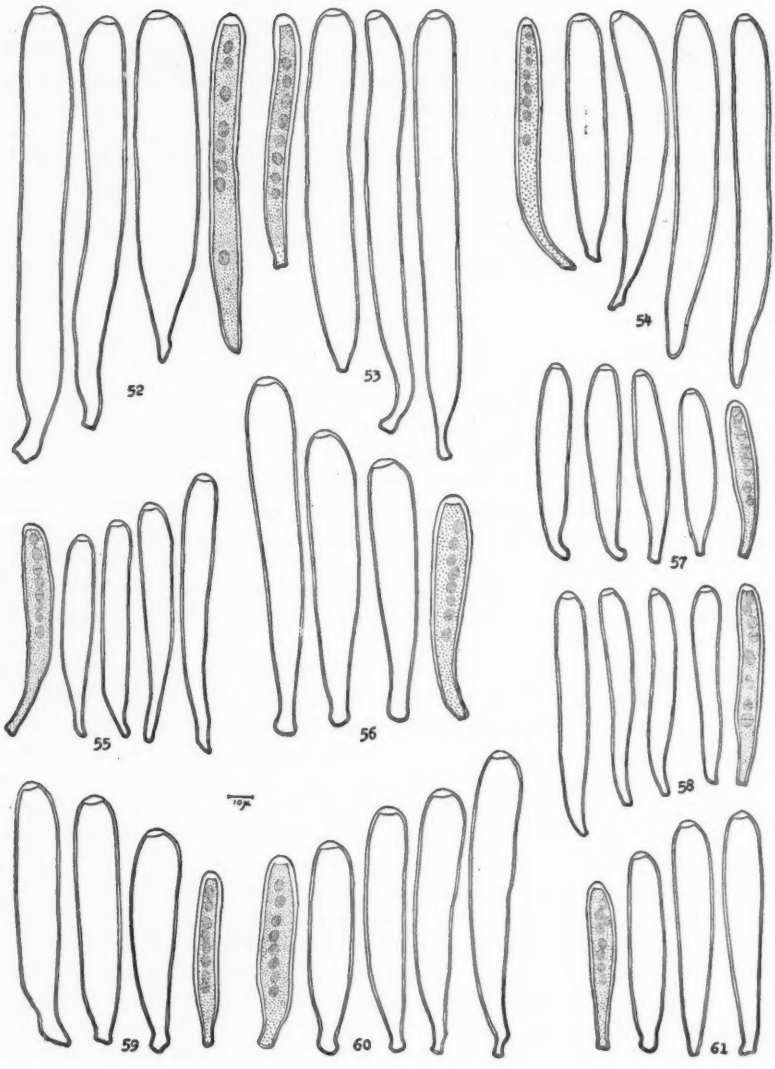
*Tympanis saligna* is the species on which Tode (1790 (41) ) founded the genus *Tympanis* but it seems to be impossible to determine with certainty the fungus that Tode had. Fries (1822 (6) ) recognized Tode's species and a search was made in the herbarium at Uppsala to determine whether any of Fries' specimens could be regarded as type. Only two specimens under this name that Fries might have examined were found. Both of these were collected by Wahlenberg in 1802 and one proved to be an old tremellaceous fungus whereas the other is an old pyrenomycete. Therefore, it is necessary to choose a neotype and this should be done in such a way as to fix the name in the sense of established usage.

This investigation has shown that two species of *Tympanis* occur on *Salix* and also that *T. spermatiospora*, which commonly occurs on *Populus*, apparently may occasionally be found on *Salix*. Obviously *T. spermatiospora* should be eliminated from consideration. The two *Salix*–inhabiting species can be readily separated both by their gross appearance and by the size of the asci. One species is densely grayish-pruinose, immersed in the bark or only slightly erumpent, and has large asci mostly exceeding 150$\mu$ in length. The other species is black, more strongly erumpent and has asci mostly less than 135$\mu$ in length.

Overholts (1933 (25) ) reported the pruinose species under the name of *T. saligna* Tode but noted that other descriptions called for smaller asci. Saccardo (1889 (34) ) described the asci as 90-110 $\times$ 15-18$\mu$ and from the description of the apothecia as "*atris, nitidulis*" it seems probable that he had the other species. Rehm (1889 (30) ) described the grayish-pruinose species but appears to have copied Saccardo's measurements of the asci, for the Rehm specimens in the herbarium of the Natural History Museum, Stockholm, have the typical large asci.

Most of the specimens that have been seen in herbaria under the name of *T. saligna* have been the grayish-pruinose species. Apart from the two Duchesnay collections, only one specimen of the other species has been seen. It is, therefore, clear that the usual concept of *T. saligna* has been based on the grayish-pruinose species, and two good exsiccati have been issued under this name by Jaap and Krieger.

It seemed desirable, therefore, to choose a type that would fix the concept of *T. saligna* as the grayish-pruinose species. Rehm (1912 (31) ) has already cited Jaap Fung. Sel. *414* and specimens of this set in the Farlow Herbarium and the herbarium of the Natural History Museum, Stockholm, were examined and found to be this fungus. It was, therefore, decided to designate Jaap Fung. Sel. *414* as a neotype.




FIGS. 52-61. Drawings of asci and primary ascospores.

FIGS. 52. *T. saligna.* 53. *T. myricariae.* 54. *T. rhoina.* 55. *T. acericola.* 56. *T. oxydendri.* 57. *T. spermatiospora.* 58. *T. sorbi.* 59. *T. hydrangeae.* 60. *T. fasciculata.* 61. *T. mutata.*

It does not appear to be a very common fungus and has only been cultured from one collection made at Ottawa. No good material of the conidial state was found in any of the specimens examined but cultures produced a typical *Pleurophomella* and von Höhnel (1916 (17) ) has described what appears to be the conidial state of this fungus and named it *Pleurophomella saligna* v. Höhn.

*Tympanis myricariae* Rehm is close to *T. saligna* morphologically and these two species are not far removed from *T. conspersa* and related species.

14. TYMPANIS MYRICARIAE v. Höhn. & Rehm, Ber. Bayer. Bot. Ges. 13 : 204. 1912. (Figs. 13, 53)

*Tympanis myricariae* Rehm nom. nud. Oesterr. bot. Zeitschr. 56 : 343. 1906.

Apothecia immersed to slightly erumpent, scattered, separate, sessile, very slightly narrowed below, at first almost globose, becoming shallow dish-shaped, circular to undulate, 0.3-0.5 mm. in diameter, 0.2-0.3 mm. in height, grayish-pruinose, hard, horny in consistency, becoming more cartilaginous when moist; hymenium concave becoming plane, black, more fleshy than the excipulum, with a thick, persistently inrolled margin; tissue of the hypothecium plectenchymatous composed of interwoven, ascending, hyaline to brownish hyphae about 2-4$\mu$ in diameter, with the walls thickened and gelatinized, at the outside becoming thicker and darker forming a rindlike excipulum; asci cylindric, obtuse at the apex, narrowed below to a short stalk, at first with the walls thickened and gelatinized, becoming thinner with maturity, at first eight-spored, finally multispored, (145)-150-180-(190) $\times$ (14)-15.0-18.0$\mu$; primary ascospores hyaline, one-celled, broadly ellipsoid to subglobose, uniseriate, 4.5-5.5 $\times$ 3.5-4.5$\mu$; secondary ascospores hyaline, cylindric to allantoid, one-celled, 2.5-4.0 $\times$ 1.0-1.5$\mu$; paraphyses hyaline, filiform, septate, simple or branched, 1.5-2.0$\mu$ in diameter, the tips scarcely swollen and embedded in a brownish, gelatinous matrix forming an epithecium.

Conidial fruiting bodies not observed.

*Host:* *Myricaria germanica* (L.) Desv.

*Type:* In Herb. Bot. Mus. Stockholm. On *Myricaria germanica*, Pfitschertal, Austria.

This species is known only from the type collection which it was possible to examine through the kindness of Dr. Sten Ahlner, of the Botanical Museum, Stockholm. It is very similar both in gross appearance and in microscopic characters to *T. saligna* Tode, but the asci are slightly smaller. Since *Salix* and *Myricaria* are not closely related it was thought preferable to recognize this as a distinct species. It has not been studied in culture and no conidial fruiting bodies were found in the material examined. In the original description the asci were said to be 120-150 $\times$ 10-12$\mu$, but it would appear that these measurements were based on immature asci.

15. TYMPANIS CONSPERSA Fr., Syst. Myc. 2 : 175. 1822. (Figs. 14, 36, 62)

*Sphaeria conspersa* Fr., Vet. Akad. Handl. p. 112. 1817.

*Cenangium conspersum* Fckl., Symb. Myc. p. 271. 1870.

*Peziza pyri* Pers., Syn. Meth. Fung. p. 671. 1801.

*Tympanis pyri* Schroet., Krypt.-Fl. Schles. III, 2 : 146. 1908.

*Sphaeria aucupariae* Pers., Syn. Meth. Fung. p. 51. 1801.

*Cenangium aucupariae* Fr., Syst. Myc. 2 : 181. 1822.

*Peziza aucupariae* Pers., Mycol. Eur. 1 : 327. 1822.

*Tympanis aucupariae* Wallr., Fl. Crypt. Germ. 2 : 427. 1833.

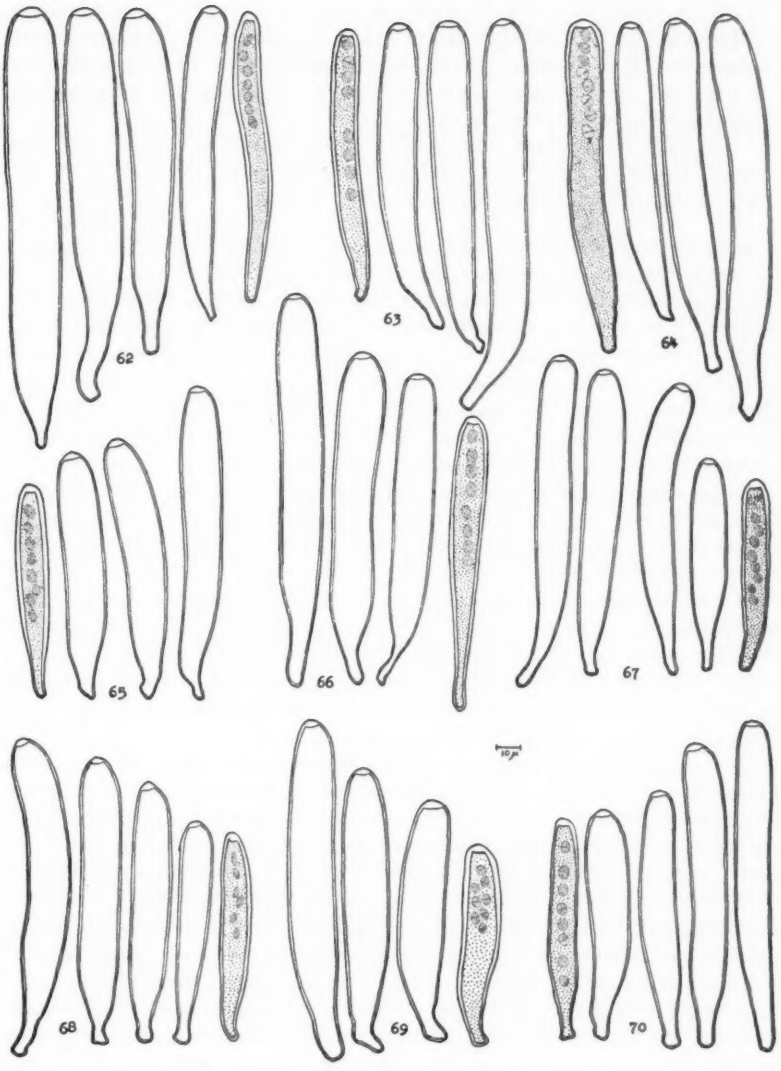
Apothecia erumpent, gregarious, usually cespitose in large clusters of up to 20 or more, occasionally single, circular, or distorted by crowding, slightly narrowed below and arising from a basal stroma, densely grayish-pruinose or becoming black and glabrous, 0.5-0.8 mm. in diameter, the clusters up to 4 mm. in diameter and 0.5-2.0 mm. in height, hard, horny in consistency, becoming more cartilaginous when moist; hymenium concave to plane, grayish-pruinose or black, more fleshy than the excipulum when moist, with a thick, persistently inrolled margin; tissue of the hypothecium plectenchymatous composed of brownish to nearly hyaline, interwoven, ascending hyphae about 2.5-3.0$\mu$ in diameter with the walls thickened and gelatinized, at the outside becoming thicker and darker forming a rindlike excipulum; asci cylindric, obtuse at the apex, narrowed below to a short stalk, at first with thick, gelatinized walls that become thinner with maturity, at first eight-spored, finally multispored, (120)-140-180-(200) $\times$ (13)-16-22-(28)$\mu$; primary ascospores hyaline, one-celled, broadly ellipsoid to subglobose, 4-8 $\times$ 3-6$\mu$; secondary ascospores hyaline, one-celled, cylindric to allantoid, 2.0-4.0 $\times$ 1.0-1.5$\mu$; paraphyses hyaline, filiform, septate, simple or branched, 1.5-2.0$\mu$ in diameter, the tips slightly swollen and embedded in a brownish, gelatinous matrix, forming an epithecium.

Conidial fruiting bodies erumpent, cespitose, in clusters of up to 20 or more, frequently on the same basal stroma as the apothecia, subglobose to ovoid densely grayish-pruinose or becoming black, about 0.2-0.3 mm. in diameter, the clusters up to 2 mm. in diameter and 0.5-1.0 mm. in height, consistency and tissue structure similar to the apothecia, containing a single globose to ovoid cavity, opening at the top; conidiophores lining the cavity, hyaline, filiform, septate, simple, not observed branching, about 15-40 $\times$ 1.5-2.0$\mu$; conidia hyaline, one-celled, cylindric to allantoid, 2.0-4.0 $\times$ 1.0-1.5$\mu$, borne at the tip and along the sides of the conidiophore.

*Host:* *Pyrus* spp., *Sorbus* spp., *Prunus spinosa* L.

*Type:* Fries Sclerom. Suec. *12.*

*Exsiccati:* Moug. & Nestl. Stirp. Crypt. Vog. *789* (as *Peziza aucupariae*); Libert Pl. Crypt. *129*; Linh. Fung. Hungar. *155* (as *T. conspersa* var. *mali*); Vize Micro-fung. Brit. *387* (*Cenangium pulveraceum*); Krieg. Fung. Sax. *2276* (as *Tympanis pyri*); Kl. Herb. Viv. Myc. *346* (as *Cenangium aucupariae*); Fckl. Fung. Rhen. *768*; Roum. Fung. Sel. Gall. Exs. *445* (as *Peziza aucupariae*); Fung. Columb. *4588*; Ell. N. Amer. Fung. *66*; Rel. Farl. *153*; Rehm Ascom. *722* (as *Tympanis conspersa* var. *mali*).




FIGS. 62-70. Drawings of asci and primary ascospores.

FIGS. 62. *T. conspersa*. 63. *T. alnea*. 64. *T. alnea* v. *hysterioides*. 65. *T. prunicola*. 66. *T. magnoliae*. 67. *T. amelanchieris*. 68. *T. salicina*. 69. *T. syringae*. 70. *T. ligustri*.

*Specimens Examined:*

CANADA: **Ontario:** Toronto, JWG *431*.

UNITED STATES: **New Hampshire:** Chocorua, FH; Crawford Ravine, FH; Swanzey, JRH *2215*. **Vermont:** Mt. Mansfield, FH. **Massachusetts:** Newton, FH; Petersham, JRH *2216*. **Connecticut:** Bethany, JWG *445*, JRH *1686*. **New York:** Ithaca, JWG *466*. **New Jersey:** Newfield, FH. **Michigan:** Rock River, Mich.

EUROPE: **Germany:** Munster, ZT *123*. **Sweden:** Stockholm, JWG *574*; Uppsala, JWG *575*; DAOM unnumbered; Handöl, JWG *960*; Uppland, UPS (on *Prunus spinosa*).

*T. conspersa* is one of the better known species of the genus but appears to be more common in Europe than in North America. The apothecia are typically densely grayish-pruinose and occur in clusters arising from a basal stroma. Conidial fruiting bodies and apothecia frequently occur on the same stroma. Some specimens are much less pruinose than others and occasional specimens are black and glabrous. It may occur on both *Pyrus* and *Sorbus* and has been reported on *Crataegus* but no specimens on this host have been seen. One collection on *Prunus spinosa* in the herbarium at Uppsala was typical *T. conspersa*.

In the original description by Fries (1822 (6) ) two specimens were cited, Fries Scler. Suec. *12* and *171*. The specimen in No. *12* is on *Sorbus* and should be recognized as type because No. *171* is on *Betula* and seems to be better placed in *T. alnea*.

In the Persoon herbarium at Leiden there is a specimen No. *910.261.34* labeled *Peziza aucupariae* Pers. It was apparently collected by Mougeot and there are notes and sketches showing asci containing four or eight ellipsoid spores and some containing many spores. The sketches depict the apothecia as more stipitate than they actually are in the specimen which is typical *T. conspersa*.

The specific epithets *conspersa* and *aucupariae* have about equal claims to recognition under the International Rules, but *conspersa* has page priority and seems to be the name more generally used. Furthermore, Fries (1822 (6) ) did not cite any specimens in the description of *Cenangium aucupariae* which would make the selection of a type more difficult if this name were accepted.

There is a group of species, including *T. alnea*, *T. amelanchieris*, *T. prunicola*, *T. magnoliae*, and *T. rhoina*, that show similarities to *T. conspersa*. They are generally cespitose, more or less grayish-pruinose and have large asci that seem to mature slowly. In most mounts from these species relatively few of the asci are fully mature and most of them are thick-walled and more slender than the fully mature asci. *T. conspersa* and *T. alnea* are very similar morphologically and are separated principally on the basis of host relationships and cultural characters.

Von Höhnel (1906 (14) ) suggested that the conidial state of *T. conspersa* had been described under various names, including *Dothiorella sorbina* Karst. *D. multiplex* (Preuss) Sacc., *D. caespitosa* (Preuss) Sacc., and *D. stromatica*

(Preuss) Sacc. Authentic material of these *Dothiorella* species has not been examined, but from the descriptions by Saccardo (1884 (33) ) this could well be so. Saccardo (1892 (35) ) suggested that *Cytosporella conspersa* Richon was the conidial state of *Tympanis conspersa* and this also may be another synonym, but no attempt has been made to find authentic material of these conidial forms

16. TYMPANIS ALNEA (Pers.) Fries, Syst. Myc. 2 : 174. 1822. (Figs. 23, 24, 63)

*Peziza alnea* Pers., Syn. Meth. Fung. p. 673. 1801.

*Cenangium alneum* Fckl., Symb. Myc. p. 271. 1870.

*Tympanis conspersa* var. *nitida* Fckl., Symb. Myc. p. 272. 1870.

*Tympanis conspersa* f. alnea Karst., Myc. Fenn. 1 : 227. 1871.

St. conid.

*Sphaeria inversa* Fr., in Kze. & Schm. Myk. Heft 2 : 36. 1823.

*Dothiorella inversa* v. Höhn., Fragm. z. Myk. No. 83. 1906.

*Pleurophomella inversa* v. Höhn., Fragm. z. Myk. No. 858. 1914.

Apothecia erumpent, gregarious, usually cespitose in clusters of 10-20 or more and up to 3 mm. in length and 0.5-2.0 mm. in height, occasionally single, circular or distorted by crowding, sessile, narrowed below to suburceolate, black, glabrous or sometimes densely grayish-pruinose, 0.3-1.0 mm. in diameter, hard, horny in consistency, becoming more cartilaginous when moist; hymenium concave to plane, black or sometimes grayish-pruinose, more fleshy than the excipulum, with a thick, more or less inrolled, persistent margin; tissue of the hypothecium plectenchymatous, composed of brownish to nearly hyaline, interwoven, ascending hyphae about 3-5$\mu$ in diameter with the walls thickened and gelatinized, at the outside becoming thicker and darker forming a rindlike excipulum; asci cylindric, obtuse at the apex, narrowed below to a short stalk, at first with thick gelatinized walls which become thinner with maturity, at first eight-spored, finally multispored, (110)-135-190-(215) $\times$ (14)-18-22-(25)$\mu$; primary ascospores hyaline, broadly ellipsoid to subglobose, one-celled, uniseriate, 5-6 $\times$ 4-5$\mu$; secondary ascospores hyaline, one-celled, cylindric to allantoid, 3-4 $\times$ 1.0-1.5$\mu$; paraphyses hyaline, filiform, septate, simple or branched, about 2-3$\mu$ in diameter, the tips slightly swollen and embedded in a brownish, gelatinous matrix, forming an epithecium.

Conidial fruiting bodies erumpent, gregarious, cespitose in clusters of up to 30 or more and up to 3 mm. in length and 1 mm. in height, occasionally single, black, glabrous or grayish-pruinose, sometimes the basal stroma only slightly developed and the pycnidia seated on it, subglobose to ovoid, 0.2-0.4 mm. in diameter, sessile or narrowed below to substipitate, sometimes with more stromatic development, forming a more or less rounded, irregular stroma with the pycnidial cavities immersed in the upper part and not in separate pycnidia; consistency and tissue structure similar to the apothecia; pycnidial cavities variable, more or less ovoid in the separate pycnidia and about 120-300$\mu$ in diameter, sometimes lobed and more irregular in the larger stromata; conidiophores lining the cavities, hyaline, filiform, simple or branched, septate,

about 20-75 × 1.5-2.0μ; conidia hyaline, one-celled, cylindric to allantoid, 3-5 × 1.0-1.5μ, borne at the tip and along the sides of the conidiophores.

*Host:* *Alnus* spp., *Betula* spp.

*Neotype:* On *Alnus incana* (L.) Moench, Mustiala, Finland. Coll. Karsten, July 1869. UPS.

*Exsiccati:* Fckl. Fung. Rhen. *768*; Rabh. Fung. Eur. *719*; Syd. Myc. Germ. *1954*; Allesch. & Schn. Fung. Bav. *264* (as *T. conspersa*); Fries Scler. Suec. *171* (as *T. conspera* on *Betula*); Jacz. Fung. Ross. *242*.

*Specimens Examined:*

CANADA: **Newfoundland:** Dec. 8, 1894, FH. **Nova Scotia:** Colch. Co., LEW *176*, JWG *525*. **Quebec:** Laurentide National Park, DAOM *5299*, JWG *617*; Bonaventure Co., DAOM *3797*; Tourville, JWG *842*; Hull, DAOM; Ironsides, DAOM. **Ontario:** Ottawa, JWG *115*; DAOM, several unnumbered specimens ex herb. John Macoun; Algonquin Park, TRT *15762*, DAOM *14854*; JWG *886*; Cobden, DAOM *4190*; Holland R. Marsh, JWG *470*; Constance Bay, DAOM *3317*, JWG *105*; Petawawa For. Exp. Stn., DAOM *7988*; DAOM *7337*, JWG *723*; JWG *728*; DAOM *15515*, JWG *820*; DAOM *19889*, JWG *858*; DAOM *19877*, JWG *859*; DAOM *5733*; Timagami For. Reserve, JWG *181*; JWG *183*; JWG *240*; JWG *249*; JWG *266*; JWG *304*; JWG *432*; JWG *482*; JWG *923*; TRT *8010*; DAOM *2521*; JRH *2207*.

UNITED STATES: **New Hampshire:** Carroll, JRH *1149*, JWG *371*; Columbia, JRH *2210*; Chocorua, FH two unnumbered specimens; North Conway, FH; Swanzey, JRH *2209*. **Vermont:** Brattleboro, FH; Middleburg, FH; Poultney, JRH *2208*. **Connecticut:** Goshen, JRH *2211*. **W. Virginia:** Morgantown, FH, WLW *2888*. **New York:** Buffalo, FH; McLean, FH, WLW *2391*; Slaterville, FH, WLW *2417*; Wilmington, JRH *2206*. **Idaho:** Bonner Co., FH ex herb. J. R. Weir *14619*. **Alaska:** FH.

EUROPE: **Germany:** FH ex herb. Barbey-Boissier *1110*. **Denmark:** Jutland, JRH *2205*; Zealand, JRH *2204*. Smolucsk, FH ex herb. hort. bot. Jurievensis *418*.

var. HYSTERIOIDES Rehm, Ber. Bay. Bot. Ges. 13 : 203. 1912. (Figs. 25, 64)

*Tympanis hysterioides* Rehm, Rabh. Kr.-Fl. 1, 3 : 268. 1889.

Apothecia black, single, circular to undulate, often more or less elongated to almost hysteriform, 0.8-2.0 mm. in diameter, 0.5-1.0 mm. in height; microscopic characters as in the typical form.

*Specimens Examined:* Type ex herb. Bot. Mus. Stockholm. On *Alnus viridis*, in Tyrol, 8/1874.

CANADA: **Nova Scotia:** Cape Breton, LEW *1239*; Truro, LEW *326*, JWG *524*. **Quebec:** Laurentide National Park, DAOM *5320*, JWG *596*. **Ontario:** Timagami For. Reserve, JWG *413*.

UNITED STATES: **New York:** Ithaca, JWG *262*.

EUROPE: **Germany:** Brandenburg, ZT.

*T. alnea* is one of the commonest species of the genus and it occurs in two forms. In the more typical form the apothecia are black and glabrous whereas in the other they are grayish-pruinose. The latter form is very similar in gross appearance to *T. conspersa* occurring on *Pyrus*, and some authors have referred this form to *T. conspersa*. However, collections of *T. alnea* have been examined in which both black and pruinose apothecia occur on the same twigs and exhibit all gradations between the two extremes. Furthermore cultures from the black and pruinose apothecia on *Alnus* resemble each other more than they do cultures from apothecia on *Pyrus* The asci of *T. conspersa* are usually a little wider than those of *T. alnea* although there is considerable overlapping in the measurements. Unquestionably the forms on *Alnus* and *Pyrus* are closely related, but I am regarding them as distinct species and considering the black and pruinose forms on *Alnus* to be variants of *T. alnea*.

Evidently a form sometimes occurs on *Betula* that has been referred to *T. conspersa*. The specimen in Fr. Scler. Suec. *171* as *T. conspersa* on *Betula* has been examined and in the absence of any clear-cut morphological characters or any cultural data it is thought preferable to refer this specimen to *T. alnea*.

The fungus was first described by Persoon (1801 (26) ) as *Peziza alnea* and was transferred to *Tympanis* by Fries (1822 (6) ). The species concept has remained relatively constant among different authors. The specimen in Rabh. Fung. Eur. *719* was in poor condition and those in Syd. Myc. Germ. *1954* and Allesch. & Schn. Fung. Bav. *264* are both the pruinose forms. The specimen in Fung. Rhen. *768* in the Farlow Herbarium is typical of the *Tympanis*, but Fuckel's description (1870 (7) ) would suggest that he saw another fungus. He stated that the spores were filiform and transferred it to *Cenangium*, claiming that it was different from the fungus in Rabh. Fung. Eur. *719*. However, the specimens examined of both these numbers contained the *Tympanis* and from the accounts of Rehm (1889 (30)) who examined Fung. Rhen. *768*, and Massee (1895 (22) ) who examined Rabh. Fung. Eur. *719*, it is evident that they also found the *Tympanis*.

Hazslinszky (1887 (12) ) also reported finding filiform ascospores in *T. alnea*, but it seems probable that another fungus has been confused with the *Tympanis*. At the Petawawa Forest Experiment Station, Ontario, a fungus has been collected that has the gross appearance of a *Tympanis* but has filiform ascospores and a *Sphaerographium*-like conidial state. This fungus is a typical *Durandiella* and will be described later in that genus.

Rehm (1889 (30)) described *T. hysterioides* which he distinguished from *T. alnea* on the basis of larger, more or less elongated apothecia usually occurring singly rather than in clusters. Later (1912 (31) ) he reduced this form to varietal rank. Through the courtesy of Dr. Sten Ahlner, of the Botanical Museum, Stockholm, it has been possible to examine Rehm's type. It agrees with specimens collected in North America and which have been studied in culture.

In spite of the striking difference in gross appearance, the asci and primary ascospores appear to be identical in this and in typical *T. alnea*. The two

forms cannot be separated culturally and, in the specimen in JWG *432*, apothecia of both types have been observed on the same twig within one centimeter of each other. Therefore it has been decided to retain this form as a variety of *T. alnea*. The *Durandiella* mentioned above is very similar in gross appearance to the var. *hysterioides* but, of course, can be readily distinguished microscopically and culturally.

Although *T. alnea* is one of the commoner species and the concept of this species has been relatively stable, it is very difficult to designate a satisfactory type. Fries (1822 (6) ) did not cite any specimens and no satisfactory material that could be designated as type was found in the herbarium at Uppsala. There were five Scandinavian collections here. One was from Lapland collected by Wahlenberg marked ex herb. Fries but was quite immature. Another Wahlenberg collection from Uppsala was Rehm's var. *hysterioides* and was not in very good condition. Another specimen collected at Uppsala and also marked ex herb. Fries was again the var. *hysterioides*. A Norwegian specimen collected by Sommerfelt was very scanty and in poor condition and also appeared to be the var. *hysterioides*. A specimen collected by Karsten in July, 1869, contained abundant material in excellent condition of both the typical black cespitose form and the var. *hysterioides*.

In the Persoon herbarium at Leiden a specimen No. *910.264.892* was marked ex herb. Fries, but I found only conidial fruiting bodies. Two other specimens, *910.265.1819* and *910.261.10*, were labeled *Peziza alnea* Pers. but were scarcely typical of the fungus. The apothecia were the size and shape of typical *T. alnea* but were mostly separate and not in the typical clusters.

It might be argued from the specimens in the herbarium at Uppsala that Fries' concept of the species was really based on the var. *hysterioides*. However, since there is no conclusive evidence that this is so, and such an interpretation would seriously upset the established nomenclature and cause confusion, it seemed desirable to choose a neotype that would fix the name in the sense of common usage. The Karsten specimen in the herbarium at Uppsala seemed the most suitable for this purpose.

Rehm (1889 (30) ) identified the conidial state that Phillips described as associated with the apothecia, as *Sphaeria inversa* Fr., and von Höhnel (1906 (14) ) transferred it to *Dothiorella* and later (1914 (15) ) to *Pleurophomella*. Dr. G. D. Darker kindly compared one of my specimens with material in the von Höhnel herbarium and considered them to be identical.

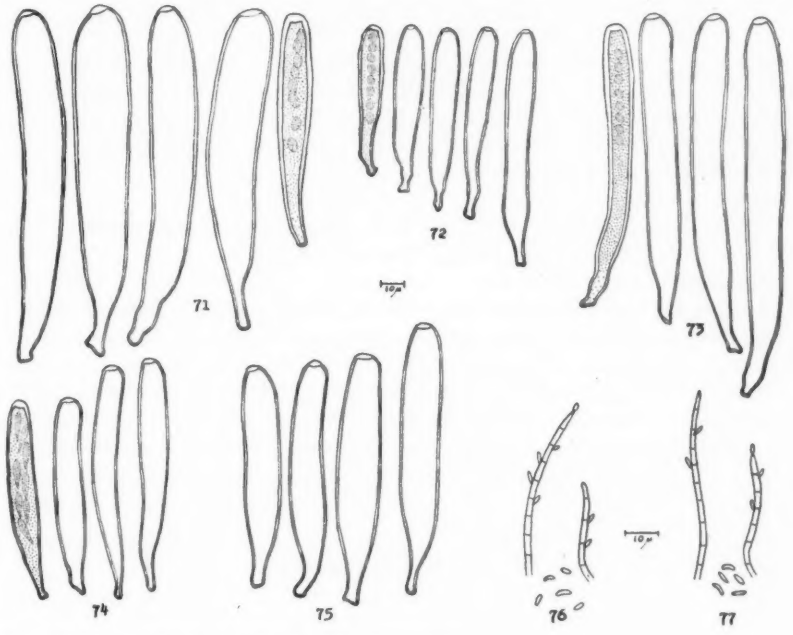
17. **Tympanis prunicola** sp. nov. (Figs. 15, 65, 76)

Apotheciis erumpentibus, dispersis vel gregariis, caespitosis ad 15 congregatis, raro solitariis, sessilibus, versus basim attenuatis, orbicularibus, undulatis vel distortis, atris, glabris vel griseo-pruinosis, 0.5-0.8 mm. diam., 0.4-1.0 mm. altis, duris, corneis in sicco, cartilagineis in humido; hymenio concavo vel plano, atro vel griseo-pruinoso, carnoso in humido, marginato; hypothecio plectenchymato; ascis cylindraceis, breve stipitatis, primo octosporis, dein multisporis, (95)-110-145-(175) × (13)-15-20-(22)$\mu$; ascosporis primariis hyalinis, ellipsoideis vel subglobosis, continuis vel uniseptatis,

uniseriatis, 5.0-7.0 × 3.0-4.0$\mu$; ascosporis secundariis hyalinis, cylindraceis vel allantoideis, unicellularis, 2.0-4.0 × 1.0-1.5$\mu$; paraphysibus hyalinis, filiformibus, septatis, simplicibus vel ramosis, 1.5-2.5$\mu$, ad apicem leviter incrassatis agglutinatisque, epithecium fuscum formantibus.

Hab.: *Prunus* spp.

Apothecia erumpent, scattered to gregarious, cespitose in clusters of up to 15, occasionally single, sessile, slightly narrowed below, circular to undulate or somewhat distorted by crowding, black, glabrous or sometimes grayish-pruinose, 0.5-0.8 mm. in diameter, 0.4-1.0 mm. in height, hard, horny in




FIGS. 71-77. Drawings of asci and primary ascospores.

FIGS. 71. *T. diospyri.* 72. *T. malicola.* 73. *T. columnaris.* 74. *T. rehmiana.* 75. *T. forsythiae.* 76. *T. prunicola,* conidiophores and conidia. 77. *T. pithya,* conidiophores and conidia.

consistency, becoming more cartilaginous when moist; hymenium concave to plane, black or grayish-pruinose, more fleshy than the excipulum, with a thick, persistent, somewhat inrolled margin; tissue of the hypothecium plectenchymatous, composed of interwoven, ascending, brownish to nearly hyaline hyphae about 2-4$\mu$ in diameter, with the walls thickened and gelatinized, at the outside becoming thicker and darker forming a rindlike excipulum; asci cylindric, obtuse at the apex, narrowed below to a short stalk, at first with the walls thickened and gelatinized, becoming thinner with

maturity, at first eight-spored, finally multispored, (95)-110-145-(175) × (13)-15-20-(22)$\mu$; primary ascospores hyaline, ellipsoid to subglobose, one- or two-celled, uniseriate, 5.0-7.0 × 3.0-4.0$\mu$; secondary ascospores hyaline, cylindric to allantoid, one-celled, 2.0-4.0 × 1.0-1.5$\mu$; paraphyses hyaline, filiform, septate, simple or branched, 1.5-2.5$\mu$ in diameter, the tips slightly swollen and embedded in a brownish, gelatinous matrix, forming an epithecium.

Conidial fruiting bodies erumpent, scattered, cespitose in clusters of up to 10, occasionally single, sometimes on the same stroma as the apothecia, sometimes appearing as ovoid to flask-shaped pycnidia, narrowed below to substipitate and seated on a basal stroma, sometimes almost entirely immersed in the basal stroma and appearing as rounded to conical elevations opening at the top, black or grayish-pruinose, about 0.1-0.3 mm. in diameter, similar in consistency and tissue structure to the apothecia, containing an ovoid or somewhat elongated cavity; conidiophores hyaline, filiform, septate, simple or occasionally branched, lining the cavity, 20-50 × 1.5-2.0$\mu$; conidia hyaline, cylindric to allantoid, one-celled, 2.0-4.0 × 1.0-1.5$\mu$, borne at the tip and along the sides of the conidiophore.

*Host:* *Prunus* spp.

*Type:* DAOM *23806*. On *Prunus* sp., Bear Island, L. Timagami, Ont. Coll. J. W. Groves, Aug. 27, 1935. Co-type, JWG *405*.

*Specimens Examined:*

CANADA: **Nova Scotia:** Kentville, DAOM *4698*, JWG *550*. **Quebec:** Ile Jesu, DAOM *7706*, JWG *731*; St. Aubert, JWG *839*; St. Elzear, DAOM *3790*. **Ontario:** Merivale, DAOM *5738*, JWG *651*; Timagami Forest Reserve, TRT *5947*, JWG *155*; TRT *6982*, JWG *182*; JWG *258*; TRT *6984*, JWG *284*; TRT *9678*, FH; JRH *1137*.

UNITED STATES: **Connecticut:** E. Granby, JRH *2549*; JRH 3034.

Four species of *Tympanis* have been reported on *Prunus*, *T. plicato-crenata* (Schw.) Fr., *T. prunastri* (Pers.) Wallr., *T. conspersa* Fr., and *T. prunastri* (Fckl.) Rehm. The identity of *T. plicato-crenata* is in doubt. Saccardo (1889 (34) ) stated that it is a synonym of *Cenangium andromedae* (Schw.) Fr. The only specimen under this name that has been examined was one in the Kew Herbarium of which the label lacked any other data. This specimen belonged in the Patellariaceae and was not close to *Tympanis*. The original description does not suggest a *Tympanis*, hence this name has been excluded for this fungus.

*T. prunastri* Wallr. is a synonym of *Dermea prunastri* (Pers.) Fr.

Undoubtedly the name *T. conspersa* has been applied to this fungus by various collectors, but *T. conspersa* differs in its cultural characters, in having larger asci and more cespitose apothecia, and in its occurrence on *Pyrus* and *Sorbus*, although one collection on *Prunus spinosa* that was examined in the Uppsala herbarium appeared to be typical *T. conspersa*.

Rehm (1889 (30) ) described a *Tympanis* on *Prunus* which he found in a specimen of Fuckel's Fungi Rhenani *1126*. This specimen was issued as *Cenangium prunastri* Fr. (= *Dermea prunastri* (Pers.) Fr.) and Rehm wrote

his description of the *Tympanis* under the name *T. prunastri* (Fckl.). However, it is clear from Fuckel's description (1870 (7) ) that he had seen the *Dermea*. Two other specimens under this number in the Farlow Herbarium and the Kew Herbarium that have been examined actually contain only the *Dermea*. Dr. Sten Ahlner, of the Botanical Museum, Stockholm, has informed me (*in litt.*) that he was unable to find a specimen of *T. prunastri* in the Rehm Herbarium. Therefore, since Rehm's name is invalid, it seemed desirable to redescribe and typify the species rather than give it a new name based on a type which has not been seen and could not be located.

This species belongs to the "conspersa" group, but the asci are smaller than those of *T. conspersa*. The clusters of apothecia are smaller and more scattered with fewer apothecia in each group. In these respects it more closely resembles *T. amelanchieris*, but it differs culturally from this species as well as from *T. conspersa*.

The conidial state is a typical *Pleurophomella*. It is possible that it has been called *Dothiorella stromatica* (Preuss) Sacc., but this has not been established.

18. **Tympanis amelanchieris** sp. nov. (Figs. 18, 67)

Apotheciis erumpentibus, gregariis vel dispersis, caespitosis, raro solitariis, sessilibus, orbicularibus vel undulatis, 0.4-1.0 mm. diam., 0.3-0.5 mm. altis, griseo-pruninosis vel atris, duris, corneis in sicco, cartilagineis in humido; hypothecio plectenchymato; ascis cylindraceis, breve stipitatis, primo 4-8-sporis, dein multisporis, (90)-110-150-(160) × (14)-15-17$\mu$; ascosporis primariis hyalinis, unicellularis, late ellipsoideis vel subglobosis, uniseriatis, 4.0-5.0 × 3.0-4.0$\mu$; ascosporis secundariis hyalinis, unicellularis, cylindricis vel allantoideis, 2.5-4.0 × 1.0-1.5$\mu$; paraphysibus hyalinis, filiformibus, septatis, simplicibus vel ramosis, 1.5-2.0$\mu$ diam., ad apicem leviter incrassatis, agglutinatisque, epithecium fuscum formantibus.

Hab.: *Amelanchier* spp.

Apothecia erumpent, gregarious to scattered, cespitose in small clusters of two to eight, occasionally single, sessile, slightly narrowed below, circular or undulate, 0.4-1.0 mm. in diameter, 0.3-0.5 mm. in height, usually grayish-pruinose, sometimes black and glabrous, hard, horny in consistency, becoming more cartilaginous when moist; hymenium concave to plane, black or grayish-pruinose, more fleshy than the excipulum, with a thick, raised, persistently inrolled margin; tissue of the hypothecium plectenchymatous, composed of interwoven, ascending, brownish to nearly hyaline hyphae about 2.0-4.0$\mu$ in diameter with the walls thickened and gelatinized, at the outside thicker and darker forming a rindlike excipulum; asci cylindric, obtuse at the apex, narrowed below to a short stalk, at first with the walls thickened and gelatinized, becoming thinner with maturity, at first four- to eight-spored, finally multispored, (90)-110-150-(160) × (14)-15-17$\mu$; primary ascospores hyaline, one-celled, broadly ellipsoid to subglobose, uniseriate, 4.0-5.0 × 3.0-4.0$\mu$; secondary ascospores hyaline, one-celled, cylindric to allantoid, 2.5-4.0 × 1.0-1.5$\mu$; paraphyses hyaline, filiform, septate, simple or branched, 1.5-2.0$\mu$

in diameter, the tips slightly swollen and embedded in a brownish, gelatinous matrix, forming an epithecium.

Conidial fruiting bodies usually arising on the same stroma as the apothecia, black or grayish-pruinose, ovoid, 100-200$\mu$ in diameter, containing an ovoid cavity, opening at the top, consistency and tissue structure similar to the apothecia; conidiophores lining the cavity, hyaline, filiform, septate, simple, not observed branching, about 25-40 $\times$ 2.0-2.5$\mu$; conidia hyaline, one-celled, cylindric to allantoid, 2.5-4.0 $\times$ 1.0-1.5$\mu$, borne at the tip and along the sides of the conidiophore.

*Host:* *Amelanchier* spp.

*Type:* DAOM *7650*, on *Amelanchier* sp., Mason Lake, Petawawa Forest Experiment Station, Ontario. Coll. I. L. Conners and J. W. Groves, Sept. 2, 1941. Co-type JWG *716*.

*Specimens Examined:*

UNITED STATES: **Idaho:** Bonner Co., JWG *751*, UIFP *1897*; JWG *752*, UIFP *1899*C; JWG *753*, UIFP *1900*.

This species is allied to *Tympanis conspersa* but differs in the smaller asci and also to some extent in gross appearance as well as in cultural characters. The apothecia are grayish-pruinose as in *T. conspersa*, but in *T. amelanchieris* there are rarely more than six in a cluster and the individual apothecia are a little larger. In the specimens examined the apothecia were found only on very small twigs.

Reports of *Cenangium aucupariae* Pers. on *Amelanchier* may refer to this fungus, but *C. aucupariae* is considered to be a synonym of *T. conspersa*. No other *Tympanis* has been described on *Amelanchier* as far as could be determined.

Only one collection, the type, has been studied in fresh condition and cultured, but the western specimens sent by Dr. John Ehrlich agreed morphologically. Both conidial fruiting bodies and apothecia have been produced on twigs of *Amelanchier* in culture.

19. **Tympanis magnoliae** sp. nov. (Figs. 22, 66)

Apotheciis erumpentibus, gregariis, caespitosis, sessilibus, versus basim attenuatis, orbicularibus vel undulatis, 0.3-0.5 mm. diam., 0.3-1.0 mm. altis, atris vel griseo-pruinosis, duris, corneis in sicco, cartilagineis in humido; hymenio concavo vel plano, atro, carnoso in humido, marginato; ascis cylindraceis, breve stipitatis, primo octosporis, dein multisporis, (130)-145-160-(180) $\times$ (16)-18-22$\mu$; ascosporis primariis ellipsoideis vel subglobosis, hyalinis, unicellularis, 6-8 $\times$ 4-6$\mu$; ascosporis secundariis hyalinis, unicellularis, cylindricis vel allantoideis, 3.0-5.0 $\times$ 1.0-1.5$\mu$; paraphysibus hyalinis, filiformibus, septatis, simplicibus, 1.5-2.0$\mu$ diam., ad apicem leviter incrassatis agglutinatisque, epithecium fuscum formantibus.

Hab.: *Magnolia* sp.

Apothecia erumpent, gregarious, cespitose with up to 12 in a cluster, sessile, narrowed below, circular or undulate, 0.3-0.5 mm. in diameter, 0.3-1.0 mm.

in height, black or grayish-pruinose, hard, horny in consistency, becoming more cartilaginous when moist; hymenium concave to plane, black, more fleshy than the excipulum, with a thick, raised margin; asci cylindric, obtuse at the apex, narrowed below to a short stalk, at first with the walls thickened and gelatinized, becoming thinner with maturity, at first eight-spored, finally multispored, (130)-145-160-(180) × (16)-18-22$\mu$; primary ascospores broadly ellipsoid to subglobose, one-celled, hyaline, uniseriate, 6-8 × 4-6$\mu$; secondary ascospores hyaline, cylindric to allantoid, one-celled, 3.0-5.0 × 1.0-1.5$\mu$; paraphyses hyaline, filiform, septate, simple, not observed branching, 1.5-2.0$\mu$ in diameter, the tips slightly swollen and embedded in a brownish, gelatinous matrix, forming an epithecium.

*Host:* *Magnolia* sp.

*Type:* FH, on *Magnolia*, Newfield, N.J. Coll. & Det. J. B. Ellis (as *Cenangium magnoliae* B. & C.) ex herb. Ellis.

*Specimens Examined:*

UNITED STATES: **New Jersey:** Vineland, FH.

Only two extremely scanty specimens of this fungus have been seen, both of them Ellis collections in the Farlow Herbarium. Dr. Donald P. Rogers has very kindly searched in the Ellis Collection at New York but was unable to find any more material.

The collection designated as the type was labeled *Cenangium magnoliae* B. & C. but the latter is a very different fungus. The second collection, of which the mount examined was slightly immature, was labeled *Tympanis viridans* but this name was never published.

Since the material was very scanty none was taken for sectioning, but this is not considered to be a very serious omission because the tissue structure of all the *Tympanis* species studied is very uniform in character and does not show distinctive features in different species. A few small conidial fruiting bodies that appeared to be a typical *Pleurophomella* were observed on the same stroma as the apothecia, but they were too few to serve as a basis for a description.

The fungus resembles *T. conspersa* somewhat in gross appearance and the asci also suggest relationship with this species. It would be desirable to study fresh material.

20. **Tympanis rhoina** sp. nov. (Figs. 17, 54)

Apotheciis erumpentibus, dispersis vel gregariis, caespitosis, raro solitariis, sessilibus, orbicularibus vel undulatis, 0.4-0.8 mm. diam., 0.3-0.6 mm. altis, atris vel griseo-pruinosis, duris, corneis in sicco, cartilagineis in humido; hymenio concavo vel plano, griseo-pruinoso, carnoso in humido; hypothecio plectenchymato; ascis cylindraceis, breve stipitatis, primo tetrasporis vel octosporis, dein multisporis, (100)-120-150-(165) × (14)-15-17-(19)$\mu$; ascosporis primariis hyalinis, unicellularis, ellipsoideis vel ovoideis, uniseriatis, 4-6 × 3-4$\mu$; ascosporis secundariis hyalinis, unicellularis, cylindricis vel allantoideis, 2.0-4.0 × 1.0-1.5$\mu$; paraphysibus hyalinis, filiformibus,

septatis, simplicibus vel ramosis, 1.5-2.0$\mu$ diam., ad apicem leviter incrassatis agglutinatisque, epithecium fuscum formantibus.

Hab.: *Rhus typhina* Torner

Apothecia erumpent, scattered to gregarious, usually cespitose in clusters of 2-10, occasionally single, sessile, narrowed below, circular or undulate, 0.4-0.8 mm. in diameter, 0.3-0.6 mm. in height, black or grayish-pruinose, hard, horny in consistency, becoming more cartilaginous when moist; hymenium concave to plane, grayish-pruinose, more fleshy than the excipulum, at first with a slightly raised margin which may disappear; tissue of the hypothecium plectenchymatous, composed of interwoven, ascending, brownish to nearly hyaline hyphae about 2-3$\mu$ in diameter, with the walls thickened and gelatinized, at the outside becoming thicker and darker forming a rindlike excipulum; asci cylindric, obtuse at the apex, narrowed below to a short stalk, at first with the walls thickened and gelatinized, becoming thinner with maturity, at first four- to eight-spored, finally multispored (100)-120-150-(165) $\times$ (14)-15-17-(19)$\mu$; primary ascospores hyaline, one-celled, ellipsoid to ovoid, uniseriate, 4-6 $\times$ 3-4$\mu$; secondary ascospores hyaline, one-celled, cylindric to allantoid, 2.0-4.0 $\times$ 1.0-1.5$\mu$; paraphyses hyaline, filiform, septate, simple or branched, 1.5-2.0$\mu$ in diameter, the tips slightly swollen and embedded in a brownish, gelatinous matrix, forming an epithecium.

Conidial fruiting bodies arising from the same stroma as the apothecia, black or grayish-pruinose, ovoid to conic, about 100-150$\mu$ in diameter, consistency and tissue structure similar to the apothecia, containing a single, ovoid cavity, opening at the top; conidiophores lining the cavity, hyaline, filiform, septate, simple, not observed branching, 15-30 $\times$ 2.0-2.5$\mu$; conidia hyaline, one-celled, cylindric to allantoid, 2.0-4.0 $\times$ 1.0-1.5$\mu$, borne at the tip and along the sides of the conidiophore.

*Host:* *Rhus typhina* Torner.

*Type:* FH unnumbered, Chocorua, New Hampshire, Sept. 30, 1902.

*Specimens Examined:*

UNITED STATES: **New Hampshire:** Chocorua, Sept. 2, 1906, FH.

This species is known only from the two specimens in the Farlow Herbarium. It has not been seen in the fresh condition and has not been studied in culture. Very few conidial fruiting bodies were present on the material examined. It resembles *T. conspersa* in gross appearance and belongs to the "conspersa" group, but the asci are slightly smaller than in *T. conspersa*.

A specimen in Roum. Fung. Sel. Gall. Exs. *1272* labeled *T. fraxini* f. *rhoidis* and said to be on *Rhus* was examined in herb. DAOM. It was very scanty and in poor condition but did appear to be the conidial state of a *Tympanis*. It might be *T. rhoina* but its identity could not be established from the material examined.

21. **Tympanis diospyri** sp. nov. (Figs. 35, 71)

Apotheciis erumpentibus, gregariis, solitariis vel subcaespitosis, sessilibus, versus basim attenuatis, orbicularibus vel undulatis, 0.5-1.0 mm. diam.,

0.3-0.5 mm. altis, atris, duris, corneis in sicco, cartilagineis in humido; hymenio concavo vel plano, atro, carnoso in humido; hypothecio plectenchymato; ascis cylindraceis, breve stipitatis, primo octosporis, dein multisporis, (125)-145-165 × 19-22$\mu$; ascosporis primariis hyalinis, unicellularis, subglobosis, uniseriatis, 4-5 × 3.5-4.5$\mu$; ascosporis secundariis hyalinis, unicellularis, cylindraceis vel allantoideis, 2.0-3.0 × 1.0-1.5$\mu$; paraphysibus hyalinis, filiformibus, septatis, simplicibus vel ramosis, 1.5-2.0$\mu$ diam., ad apicem leviter incrassatis agglutinatisque, epithecium fuscum formantibus.

Hab.: *Diospyros virginiana* L.

Apothecia erumpent, gregarious, separate or in small clusters of up to about six, sessile, slightly narrowed below to somewhat turbinate, circular or undulate, 0.5-1.0 mm. in diameter, 0.3-0.5 mm. in height, black, glabrous, hard, horny in consistency, becoming more cartilaginous when moist; hymenium concave to plane, black, more fleshy than the excipulum, with a thick, raised, and slightly inrolled margin; tissue of the hypothecium plectenchymatous, composed of brownish to hyaline, interwoven, ascending hyphae about 1.5-2.5$\mu$ in diameter with the walls thickened and gelatinized, at the outside becoming thicker and darker forming a rindlike excipulum; asci cylindric, obtuse at the apex, narrowed below to a short stalk, at first with the walls thickened and gelatinized, becoming thinner with maturity, at first eight-spored, finally multispored, (125)-145-165 × 19-22$\mu$; primary ascospores hyaline, one-celled, subglobose, uniseriate, 4-5 × 3.5-4.5$\mu$; secondary ascospores hyaline, one-celled, cylindric to allantoid, 2.0-3.0 × 1.0-1.5$\mu$; paraphyses hyaline, filiform, septate, simple or branched, about 1.5-2.0$\mu$ in diameter, the tips slightly swollen and embedded in a brownish, gelatinous matrix, forming an epithecium.

Conidial fruiting bodies erumpent, gregarious, separate or in clusters of up to about six, black, glabrous, ovoid to flask-shaped, 0.1-0.3 mm. in diameter, 0.2-0.6 mm. in height, consistency and tissue structure similar to the apothecia, containing an ovoid cavity opening at the top; conidiophores lining the cavity, hyaline, filiform, septate, not observed branching, 10-60 × 1.5-2.0$\mu$; conidia hyaline, one-celled, cylindric to allantoid, 2.0-3.0 × 1.0-1.5$\mu$, borne at the tip and along the sides of the conidiophore.

*Host:* *Diospyros virginiana* L.

*Type:* DAOM *23429*, Agric. Campus, Athens, Ga. Coll. Todd and Miller, Dec. 5, 1948. Co-type JWG *924*.

This species is known only from a single collection sent by Dr. J. H. Miller from Georgia. The specimen was in excellent condition and cultures were obtained from both ascospores and conidia. As far as could be ascertained there is no previous report of a *Tympanis* occurring on *Diospyros*. The large asci and rather persistently inrolled margin suggest its relationship to the "conspersa" group, but it is less cespitose and the conidial fruiting bodies are more scattered than in other species of this group.

22. TYMPANIS OXYDENDRI Ell. & Ev. Proc. Acad. Philad. 46: 352. 1894. (Figs. 26, 56.)

Apothecia erumpent, scattered, separate or cespitose in small clusters of two to six, sessile, narrowed below, circular or undulate, 0.4-0.8 mm. in diameter, 0.3-0.5 mm. in height, black, glabrous, hard, horny in consistency, becoming more cartilaginous when moist; hymenium plane or slightly convex, more fleshy than the excipulum, black, at first with a slightly raised margin which later may disappear; tissue of the hypothecium plectenchymatous, composed of interwoven, ascending, brownish to nearly hyaline hyphae about 3-5$\mu$ in diameter with the walls thickened and gelatinized, at the outside becoming thicker and darker forming a rindlike excipulum; asci cylindric, obtuse at the apex, narrowed below to a short stalk, at first with the walls thickened and gelatinized becoming thinner with maturity, at first eight-spored, finally multispored, (110)-120-150-(165) $\times$ (16.5)-18.0-22.0-(23.0)$\mu$; primary ascospores hyaline, one-celled, broadly ellipsoid to subglobose, uniseriate, 5.0-8.0 $\times$ 4.5-5.0$\mu$; secondary ascospores hyaline, one-celled, cylindric to allantoid, 3.0-5.0 $\times$ 1.0-1.5$\mu$; paraphyses hyaline, filiform, septate, simple or branched, 1.5-2.0$\mu$ in diameter, the tips scarcely swollen and embedded in a brownish, gelatinous matrix forming an epithecium.

Conidial fruiting bodies erumpent, cespitose or occasionally single, black, glabrous, ovoid to conic, about 0.2-0.3 mm. in diameter, the clusters up to 0.5 mm. in height, consistency and tissue structure similar to the apothecia, containing a single, subglobose to ovoid cavity, opening at the top; conidiophores lining the cavity, hyaline, filiform, septate, simple, not observed branching, about 20-50 $\times$ 2.0-2.5$\mu$; conidia hyaline, one-celled, cylindric to allantoid, 3.0-5.0 $\times$ 1.0-1.5$\mu$, borne at the tip and along the sides of the conidiophore.

*Host:* *Oxydendrum arboreum* (L.) DC.

*Type:* On dead limbs of *Oxydendrum arboreum*, Fayette Co., W. Virginia, Coll. L. W. Nuttall, Dec. 12, 1893. No. *1296*, J. B. E. *258*. Portion in the Farlow Herbarium examined.

*Exsiccati:* Ell. & Ev. Fung. Columb. *247*; *1534* (as *Godronia rugosa*)

*Specimens Examined:*

UNITED STATES: **Georgia:** Athens, JWG *829*; JWG *922* (conidial stage only).

In this species the apothecia are rather small and not strongly erumpent. It seems to be fairly close to *T. syringae*. Cultures have been obtained from the Georgia specimens kindly sent by Dr. J. H. Miller.

23. TYMPANIS SYRINGAE Fuckel, Fung. Rhen. *2144*. 1867. (Figs. 27, 69)

*Cenangium syringae* Fckl. Symb. Myc. p. 270. 1870.

Apothecia slightly erumpent, scattered to gregarious, separate or sometimes subcespitose with two or three together, circular to undulate, sessile, slightly narrowed below, 0.4-0.8 mm. in diameter, 0.2-0.3 mm. in height, black, glabrous or slightly grayish-pruinose, hard, horny in consistency, becoming

more cartilaginous when moist; hymenium concave to plane, black or greenish when moist, more fleshy than the excipulum, at first with a slightly raised margin which later may disappear; tissue of the hypothecium plectenchymatous, composed of interwoven, ascending, brownish to nearly hyaline hyphae about 2-3$\mu$ in diameter, with the walls thickened and gelatinized, at the outside becoming thicker and darker forming a rindlike excipulum; asci cylindric, obtuse at the apex, narrowed below to a short stalk, at first with the walls thickened and gelatinized, becoming thinner with maturity, at first eight-spored, becoming multispored, (95)-110-130-(150) × (15)-18-20-(23)$\mu$; primary ascospores hyaline, broadly ellipsoid to ovoid or subglobose, one- or two-celled, uniseriate to irregularly biseriate, about 3-6 × 3.0-4.5$\mu$; secondary ascospores hyaline, one-celled, cylindric to allantoid, 2.0-4.0 × 1.0-1.5$\mu$; paraphyses hyaline, filiform, septate, simple or branched, 2.0-2.5$\mu$ in diameter, the tips slightly swollen and embedded in a gelatinous matrix, forming an epithecium.

*Host:* *Syringa vulgaris* L.

*Type:* Fuckel, Fung. Rhen. *2144.*

*Exsiccati:* Rehm Ascom. *864.*

*Specimens Examined:*

EUROPE: **Germany:** ex Herb. Barbey-Boissier *1100*, FH.

The specimen issued in Fuckel's Fungi Rhenani as *Tympanis syringae* was accompanied by a description on the label and can, therefore, be regarded as a valid publication. Fuckel later (1870 (7) ) described it under *Cenangium.* Two specimens of Fung. Rhen. *2144* have been examined, one in the Farlow Herbarium, the other in the Kew Herbarium. The Kew specimen was immature, but the Farlow specimen was in good condition. The specimens of Rehm Ascom. *864* in the Farlow Herbarium and in the Zurich Herbarium were also examined and proved to be good specimens of the same fungus.

Rehm (1889 (30), 1912 (31) ) apparently confused this species with another fungus that had been described by Winter (1874 (46) ) as *Cenangium syringae* and said to have *Tympanis*-like apothecia and elongate, septate ascospores. Rehm thought these might be the primary ascospores of *T. syringae,* but I have found ellipsoid to subglobose primary ascospores in *T. syringae* and it is probable that Winter's fungus was a *Corynella* or *Durandiella.*

As far as known, this species has not been reported from North America. No fresh material has been seen and it has not been studied in culture. No conidial fruiting bodies were found in the material examined.

24. TYMPANIS LIGUSTRI Tulasne, Sel. Fung. Carp. 3 : 154. 1865. (Figs. 20, 70)

*Cenangium ligustri* Fckl., Symb. Mycol. p. 268. 1870.

*Tympanis saligna* f. *ligustri* Fckl., Fung. Rhen. *767.* 1863.

*Tympanis ligustri* var. *japonica* Sacc., Mich. 1 : 58. 1877.

Apothecia not strongly erumpent, gregarious, separate or sometimes in small clusters, circular or undulate, sessile, narrowed below, 0.4-0.8-(1.0) mm. in diameter, 0.2-0.4 mm. in height, black, glabrous, hard, horny in consistency,

becoming more cartilaginous when moist; hymenium concave to plane, black or greenish when moist, more fleshy than the excipulum, at first with a thick, raised margin which later may disappear; tissue of the hypothecium plectenchymatous composed of interwoven, ascending, brownish to nearly hyaline hyphae about 1.5-3.0$\mu$ in diameter with the walls thickened and gelatinized, becoming thicker and darker at the outside forming a rindlike excipulum; asci cylindric, obtuse at the apex, narrowed below to a short stalk, at first with the walls thickened and gelatinized, becoming thinner with maturity, at first eight-spored, finally multispored, (100)-110-140-(155) $\times$ (12)-15-18-(21)$\mu$; primary ascospores hyaline, broadly ellipsoid to ovoid or subglobose, one- or two-celled, uniseriate, about 4-6 $\times$ 4.5$\mu$ secondary ascospores hyaline, one-celled, cylindric to allantoid, 3-4 $\times$ 1.0-1.5$\mu$; paraphyses hyaline, filiform, septate, simple or branched, about 1.5-2.0$\mu$ in diameter, the tips slightly swollen and embedded in a brownish, gelatinous matrix forming an epithecium.

Conidial fruiting bodies not observed.

*Host:* *Ligustrum vulgare* L., *L. japonicum* Thunberg.

*Type:* In Mus. Hist. Nat., Paris, *in ramulis Ligustri*, Feb. 8, 1853.

*Exsiccati:* Rabenh. Fung. Eur. *229* (as *T. saligna* Tode); Fckl. Fung. Rhen. *767* (as *T. saligna* f. *ligustri*).

*Specimens Examined:*

EUROPE: **Switzerland:** Bern, ZT; Neuchatel, FH ex herb. Barbey-Boissier *1138*. **Germany:** S coll. Rehm 8/1872 (as *T. saligna*). **Austria:** Nassau, FH ex herb. Barbey-Boissier *1105*. **Italy:** Coll. Bresadola, UPS.

The fungus was originally described by Tulasne (1865 (43) ) and the type in the herbarium of the Museum of Natural History, Paris, has been examined. The asci were said to be 95-112 $\times$ 16$\mu$ in the original description, but in the mount examined they were 100-135 $\times$ 15-18$\mu$. It seems from the specimens examined that the asci remain for a long time in a partially mature condition in which the secondary ascospores have formed, but the walls of the asci are still thick and the diameter of the asci about 12-16$\mu$. In the Zurich material fully mature asci were found with thin walls and the diameter reaching 21$\mu$ although the thick-walled, narrower asci predominated in the mount. It would appear that most of the published measurements of the asci of this species have been based on these immature asci.

Phillips (1893 (28) ) and Massee (1895 (22) ) reported that there were *Diplodia*-like conidia mixed among the asci and paraphyses, but these were not observed in any material examined by me. Tulasne (1853 (42) ), referring to an earlier report by Berkeley who claimed to have observed such spores, stated that he had been unable to find them. It seems probable, that, if such spores have been observed in British specimens, they do not belong to the *Tympanis* but rather to some other fungus, possibly a parasite.

The var. *japonica* named by Saccardo (1877 (32) ) seems to be based only on its occurrence on *Ligustrum japonicum* and slightly wider asci, 18$\mu$ in diameter. However, I have observed asci up to 21$\mu$ in diameter in specimens

on *L. vulgare* and consider that the var. *japonica* should be reduced to synonymy with the species.

As far as is known, there are no authentic records of this species in North America. In the Farlow Herbarium there is one specimen under this name said to be from Newton, Mass. It looks like a *Tympanis* but is in too poor condition to identify.

The specimen cited above in the herbarium of the Museum of Natural History, Stockholm, labeled *T. saligna* and collected by Rehm, seems to be *T. ligustri.* It is certainly neither of the species occurring on *Salix* and has the gross appearance of *T. ligustri* and agrees with it microscopically. The host is not stated and could not be determined with certainty.

25. TYMPANIS FASCICULATA Schw. Syn. Fung. Amer. bor. p. 237. 1832. (Figs. 32, 37, 39, 60)

*Tympanis rhabdospora* Berk. & Curt. Grev. 4 : 3. 1875.

*Godronia rhabdospora* Sacc. Syll. Fung. 8 : 602. 1889.

*Tympanis micheneri* Berk. & Curt. in herb.

Apothecia erumpent, gregarious to scattered, separate or cespitose in small clusters of two to six occasionally to 15, sessile, narrowed below, circular or undulate, 0.3-1.0 mm. in diameter, 0.3-0.8 mm. in height, black, glabrous, hard, horny in consistency, becoming more cartilaginous when moist; hymenium concave to plane, black or greenish when moist, more fleshy than the excipulum, at first with a thick, raised margin which later may disappear; tissue of the hypothecium plectenchymatous composed of interwoven, ascending, brownish to nearly hyaline hyphae about 3-5$\mu$ in diameter, with the walls thickened and gelatinized, at the outside becoming thicker and darker forming a rindlike excipulum; asci cylindric, obtuse at the apex, narrowed below to a short stalk, at first with the walls thickened and gelatinized, becoming thinner with maturity, at first eight-spored, finally multispored, (80)-100-130-(150) $\times$ (15)-17-22-(25)$\mu$; primary ascospores ellipsoid to ovoid or subglobose, hyaline, one- to two-celled, uniseriate, about 5-8 $\times$ 3-6$\mu$; secondary ascospores hyaline, one-celled, cylindric to allantoid, 2.0-4.0 $\times$ 1.0-1.5$\mu$; paraphyses hyaline, filiform, septate, simple or branched, 1.5-2.5$\mu$ in diameter, the tips slightly swollen and embedded in a brownish, gelatinous matrix, forming an epithecium.

Conidial fruiting bodies erumpent, gregarious, single or sometimes cespitose, cylindric to cylindric-conic or sometimes ovoid, black, glabrous, 150-400$\mu$ in diameter, 200-600$\mu$ in height, consistency, and tissue structure similar to the apothecia, containing an ovoid to somewhat elongated cavity opening at the top; conidiophores hyaline, filiform, lining the cavity, septate, simple or branched, 10-75 $\times$ 1.5-2.5$\mu$; conidia hyaline, one-celled, cylindric to allantoid, 2.0-5.0 $\times$ 1.0-1.5$\mu$, borne at the tip and along the sides of the conidiophore.

*Host:* *Viburnum* spp.

*Type:* In herb. Acad. Nat. Sci., Philadelphia.

*Exsiccati:* Rel. Farl. *154b;* Ell. N. Amer. Fung. *65;* Fung. Columb. *814.*

*Specimens Examined:*

CANADA: **Quebec:** Duchesnay, DAOM *5303*, JWG *605*; Ile Jésu, DAOM *7648*, JWG *732*; Tourville, JWG *836*. **Ontario:** Parry Sound, TRT *6937*, JWG *277*; Petawawa Forest Exp. Stn., DAOM *15511*, JWG *817*; DAOM *19838*, JWG *864*; DAOM *19656*, JWG *865*; DAOM *7323*; Algonquin Park, TRT *22333*; TRT *15754*; Timagami Forest Reserve, JRH *2219*; TRT *7207*, JWG *147*; TRT *6939*, JWG *229*; TRT *6934*, JWG *238*; TRT *7392*, JWG *252*; TRT *6938*, JWG *269*.

UNITED STATES: **Maine:** Brunswick, JRH *2222*. **New Hampshire:** Chocorua, FH. **Vermont:** Arlington, JRH *2221*; Ripton, FH. **New Jersey:** Newfield, FH. **New York:** Newcomb, JRH *2218*. **Pennsylvania:** (as *T. micheneri* B. & C.) K, BM, UPS. **New England:** (as *T. rhabdospora* B. & C.) K, UPS, P.

The *Tympanis* commonly occurring on *Viburnum* has been identified as *T. fasciculata* Schw. by several authors and, through the kindness of Dr. F. W. Pennell and Dr. S. C. Damon, it was possible to examine the type in the herbarium of the Academy of Natural Sciences in Philadelphia. The specimen said to be "*in ramorum fruticis ignoti*" consisted of two small twigs bearing a few, immature-looking apothecia. The material was not in good condition but in a mount I found a few asci with subglobose primary ascospores and a few asci with secondary ascospores that measured about 90-105 × 14-16$\mu$. Since this was small for the species on *Viburnum* it was thought essential to determine the host with certainty. Mr. J. D. Hale, wood technologist, Forest Products Laboratory, Ottawa, examined the wood and identified it as *Viburnum*.

Schweinitz (1832 (36) ) also described *Tympanis turbinata* which has been reported on *Viburnum*. The type of this species, which was also loaned from Philadelphia, proved to be a *Scleroderris* on *Diervilla*. The apothecia were immature and asci and spores were not observed but its generic position was unmistakable. A specimen in Kew Herbarium from the Schweinitz Herbarium and mounted in a type folder yielded conidia of the type usually associated with *Scleroderris* species. Farlow (Thaxter 1922 (40) ) transferred it to *Godronia* and, although it is congeneric with the type of *Scleroderris* and not with the type of *Godronia*, further nomenclatural changes may be deferred until a critical revision of this group is completed

Seaver (1945 (37) ) described *Godronia viburnicola* based on the specimen in Rel. Farl. *154a* labeled *Tympanis fasciculata* Schw. This fungus is a *Durandiella* and a specimen in the Kew Herbarium ex Schw. Herb. labeled *Tympanis fasciculata* Schw. and mounted in a type folder proved to be this fungus also. It is quite distinct by reason of its filiform ascospores and conidial state consisting of beaked pycnidia bearing elongate-fusiform to subfiliform conidia.

Therefore, since the specimen of *T. fasciculata* at Philadelphia bears the exact data as published in the original description by Schweinitz (1832 (36) ), and is unquestionably a true *Tympanis* occurring on *Viburnum*, and since no other *Tympanis* is known on *Viburnum*, it can be accepted as the type of the

species commonly found on this host. The slightly smaller asci could be explained by the immaturity and poor condition of the material.

Specimens of the *Tympanis* on *Viburnum* were also found in the herbaria at Kew, the British Museum, and Uppsala under the name *Tympanis micheneri* B. & C. but this name apparently was never published.

A puzzling situation developed with reference to *Tympanis rhabdospora* B. & C. This species was described by Berkeley (1875 (1) ) and was said to have filiform ascospores and to occur on *Acer*. In 1947 a specimen labeled "type" was examined in the Kew Herbarium. The label was in Berkeley's handwriting and agreed with the data as cited in the original description. Accompanying the specimen was a pencil sketch showing three filiform, *Scleroderris*-like spores and a broken ascus with two smilar spores protruding. However, the specimen, which consisted of a piece of bark about 2 × 1 cm., bore only four clusters of *Tympanis*-like apothecia and one immature stroma. Later study of a mount from an apothecium revealed a *Tympanis*, and it did not agree with the *Tympanis* on *Acer* but did agree with the species on *Viburnum*. In 1950 Kew Herbarium was again visited and this specimen very carefully re-examined, but no other fungus than the *Tympanis* could be found. In the herbaria at Uppsala and Paris, specimens were also found labeled *T. rhabdospora* and collected by Sprague. These specimens appeared to be duplicates of the type at Kew and mounts showed complete morphological agreement. Fortunately the specimen at Paris contained some wood and this was examined by Dr. C. G. Riley, Laboratory of Forest Pathology, Saskatoon, Sask., who stated that it was not *Acer* but matched *Viburnum*.

It is, therefore, concluded that the host in the type specimen of *T. rhabdospora* was misidentified as *Acer* and this name becomes a synonym of *T. fasciculata*. There is no explanation as to why Berkeley should have reported and drawn filiform ascospores unless a single apothecium of the above-mentioned *Durandiella* were present and he had happened to mount it. Certainly the type now consists solely of the *Tympanis*.

In culture this species grows slowly, producing whitish to yellowish yeastlike colonies with scarcely any aerial mycelium and rarely producing pycnidia. In twig culture it has produced both typical pycnidia and apothecia.

26. **Tympanis hydrangeae** sp. nov. (Figs. 28, 59)

Apotheciis erumpentibus, dispersis vel gregariis, solitariis vel subcaespitosis, sessilibus, orbicularibus vel undulatis, 0.5-0.8 mm. diam., 0.2-0.3 mm. altis, atris, duris, corneis in sicco, cartilagineis in humido; hymenio concavo vel plano, carnoso in humido; hypothecio plectenchymato; ascis cylindraceis, breve stipitatis, primo octosporis, dein multisporis, (75)-90-110-(115) × (17)-18-20-(22)$\mu$; ascosporis primariis hyalinis, continuis vel uniseptatis, late ellipsoideis vel subglobosis, 4-5 × 3-4$\mu$; ascosporis secundariis hyalinis, unicellularis, cylindricis vel allantoideis, 2.0-3.0 × 1.0-1.5$\mu$; paraphysibus hyalinis, filiformibus, septatis, simplicibus vel ramosis, 1.5-2.0$\mu$ diam; ad apicem leviter incrassatis agglutinatisque, epithecium fuscum formantibus.

Hab.: *Hydrangea* sp.

Apothecia erumpent, scattered to gregarious, separate or sometimes two or three in a cluster, sessile, slightly narrowed below, circular or undulate, 0.5-0.8 mm. in diameter, 0.2-0.3 mm. in height, black, glabrous, hard, horny in consistency, becoming more cartilaginous when moist; hymenium concave to plane or occasionally convex, black, more fleshy than the excipulum, at first with a thick, raised margin which later may disappear; tissue of the hypothecium plectenchymatous, composed of interwoven, ascending, brownish to nearly hyaline hyphae about 2-4$\mu$ in diameter with the walls thickened and gelatinized, at the outside becoming thicker and darker forming a rindlike excipulum; asci cylindric, obtuse at the apex, narrowed below to a short stalk, at first with the walls thickened and gelatinized, becoming thinner with maturity, at first eight-spored, finally multispored (75)-90-110-(115) × (17)-18-20-(22)$\mu$; primary ascospores hyaline, one- or two-celled, broadly ellipsoid to subglobose, uniseriate, about 4-5 × 3-4$\mu$; secondary ascospores hyaline, one-celled, cylindric to allantoid, 2.0-4.0 × 1.0-1.5$\mu$; paraphyses hyaline, filiform, septate, simple or branched, 1.5-2.0$\mu$ in diameter, the tips slightly swollen and embedded in a brownish, gelatinous matrix forming an epithecium.

*Host:* *Hydrangea* sp.

*Type:* University of Michigan Herbarium. On *Hydrangea* sp., Tacoma Park, Coll. C. H. Kauffman, Dec. 13, 1918. As *Tympanis turbinata* Schw.

This species is known only from the type collection. The material was rather scanty and no conidial fruiting bodies were observed on the twigs. No *Tympanis* species has been reported on *Hydrangea* as far as is known. This fungus would appear to be closely related to *T. fasciculata* by reason of the broad asci. It may be close to *T. forsythiae* also, which is somewhat similar in growth habit and also has short broad asci. However, the asci of the fungus on *Hydrangea* were broader than those of *T. forsythiae*.

27. **Tympanis forsythiae** sp. nov. (Figs. 21, 75)

Apotheciis erumpentibus, dispersis, solitariis vel subcaespitosis, sessilibus, orbicularibus vel undulatis, 0.3-0.8 mm. diam., 0.2-0.3 mm. altis, atris, duris, corneis in sicco, cartilagineis in humido; hymenio concavo vel plano, atro, carnoso in humido; hypothecio plectenchymato; ascis cylindraceis, breve stipitatis (primo octosporis?), demun multisporis, (80)-90-110-(125) × 14-17$\mu$: ascosporis primariis nondum visis; ascosporis secundariis hyalinis, unicellularis, cylindricis vel allantoideis, 2.0-4.0 × 1.0-1.5$\mu$; paraphysibus hyalinis, filiformibus, septatis, simplicibus vel ramosis, 2.0-2.5$\mu$ diam., ad apicem leviter incrassatis agglutinatisque, epithecium fuscum formantibus.

Hab.: *Forsythia* sp.

Apothecia erumpent, scattered, mostly separate or in small clusters of up to five, sessile, slightly narrowed below, circular to undulate, 0.3-0.8 mm. in diameter, 0.2-0.3 mm. in height, black, glabrous, hard, horny in consistency, becoming more cartilaginous when moist; hymenium concave to plane or

slightly convex, black, more fleshy than the excipulum, at first with a thick, raised margin which later may disappear; tissue of the hypothecium plectenchymatous, composed of interwoven, ascending, brownish to nearly hyaline hyphae about 2-4$\mu$ in diameter with the walls thickened and gelatinized, at the outside becoming thicker and darker forming a rindlike excipulum; asci cylindric, obtuse at the apex, narrowed below to a short stalk, at first with the walls thickened and gelatinized, becoming thinner with maturity (at first eight-spored ?), finally multispored, (80)-90-110-(125) $\times$ 14-17$\mu$; primary ascospores not observed; secondary ascospores hyaline, one-celled, cylindric to allantoid, 2.0-4.0 $\times$ 1.0-1.5$\mu$; paraphyses hyaline, filiform, septate, simple or branched, 2.0-2.5$\mu$ in diameter, the tips slightly swollen and embedded in a brownish, gelatinous matrix forming an epithecium.

*Host:* *Forsythia* sp.

*Type:* FH, on *Forsythia* (cult.), Newfield, N.J. ex herb. Ellis, May 5, 1886 (as *Tympanis spermatiospora* Nyl.).

The only specimen of this fungus that has been seen is in the Farlow Herbarium under the name *Tympanis spermatiospora* Nyl. The material is not very abundant and no conidial fruiting bodies were observed. In the mount examined no primary ascospores could be found. The asci are relatively short and broad but they are larger than those of *T. spermatiospora* and the apothecia are less strongly erumpent.

28. **Tympanis salicina** sp. nov. (Figs. 30, 68)

Apotheciis erumpentibus, gregariis, solitariis vel caespitosis, orbicularibus vel undulatis, sessilibus, versus basim attenuatis, 0.5-1.0 mm. diam., 0.2-0.5 mm. altis, atris, glabris, corneis in sicco, cartilagineis in humido; hymenio concavo vel plano, atro, carnoso in humido; hypothecio plectenchymato; ascis cylindraceis, breve stipitatis, primo octosporis, dein multisporis, (95)-110-135-(145) $\times$ (14)-15-17-(18)$\mu$; ascosporis primariis hyalinis, ellipsoideis vel fusoideis, unicellularis, uniseriatis, 5-6 $\times$ 2-3$\mu$; ascosporis secundariis hyalinis, cylindricis vel allantoideis, unicellularis, 2.0-4.0 $\times$ 1.0-1.5$\mu$; paraphysibus hyalinis, filiformibus, septatis, simplicibus vel ramosis, 1.5-2.0$\mu$ diam., ad apicem leviter incrassatis agglutinatisque, epithecium fuscum formantibus.

Hab.: *Salix* spp.

Apothecia erumpent, gregarious, separate or cespitose in small clusters, circular or undulate, sessile, narrowed below, 0.5-1.0 mm. in diameter, 0.2-0.5 mm. in height, black, glabrous, hard, horny in consistency becoming more cartilaginous when moist; hymenium concave to plane, black, more fleshy than the excipulum, at first with a slightly raised margin which later may disappear; tissue of the hypothecium plectenchymatous, composed of interwoven, ascending, brownish to nearly hyaline hyphae about 1.5-3.0$\mu$ in diameter with the walls thickened and gelatinized, at the outside becoming thicker and darker forming a rindlike excipulum; asci cylindric, obtuse at the apex, narrowed below to a short stalk, at first with the walls thickened and gelatinized becoming thinner with maturity, at first eight-spored, finally

multispored, (95)-110-135-(145) × (14)-15-17-(18)$\mu$; primary ascospores hyaline, one-celled, ellipsoid to fusoid, uniseriate, 5-6 × 2-3$\mu$; secondary ascospores hyaline, one-celled, cylindric to allantoid, 2.0-4.0 × 1.0-1.5$\mu$; paraphyses hyaline, filiform, septate, simple or branched, 1.5-2.0$\mu$ in diameter, the tips slightly swollen and embedded in a brownish, gelatinous matrix, forming an epithecium.

*Host:* *Salix* spp.

*Type:* DAOM *5341*, Duchesnay, Que., Aug. 25, 1938. JWG *590* (cotype).

*Specimens Examined:*

CANADA: **Quebec:** Duchesnay JWG *588*.

EUROPE: P, Rev. Bloxam donnait 1855. Herbier du Dr. Roussel acquis en 1875.

This species is known only from three collections. It was collected twice at Duchesnay, Que., during the Mycological Society Foray in 1938 and both of these collections were cultured. A specimen in the herbarium of the Museum of Natural History, Paris, labeled *T. saligna* Tode also appears to be this species. Much of the handwriting on the label could not be deciphered, but it seemed to be a specimen sent by Bloxam in 1855 probably to Dr. Roussel whose herbarium had been acquired by the Paris Museum in 1875. It was similar in gross appearance to the Duchesnay collections, but in the mount examined the asci were slightly smaller. There was, however, a very high proportion of immature asci, and it matched very well a mount of an immature apothecium from the Duchesnay material.

The gross appearance is different from *T. saligna*. It is more strongly erumpent and lacks the dense grayish-pruinose covering and persistently inrolled margin. The asci are smaller than those of *T. saligna* also, but they are considerably larger than the asci of *T. spermatiospora*, which may sometimes occur on *Salix*.

Cultures of this species grew poorly compared to those of most *Tympanis* species. No conidial fruiting bodies were found in the fresh material, but on sterilized twigs of *Salix* typical *Pleurophomella* pycnidia were produced.

29. TYMPANIS ACERICOLA Groves apud Wehm., Can. J. Research, C, 18 : 544. 1940. (Figs. 31, 55)

*Tympanis acerina* Rehm (non Peck), Nov. Közl. 4 : 4. 1905.

Apothecia erumpent, scattered to gregarious, cespitose in clusters of about two to eight, occasionally single, subsessile or very short stipitate, circular or undulate, 0.5-1.5 mm. in diameter, 0.5-1.0 mm. in height, black, glabrous, hard, horny in consistency, becoming more cartilaginous when moist; hymenium plane to convex, black, more fleshy than the excipulum, at first with a raised margin which later may disappear; tissue of the hypothecium plectenchymatous, composed of interwoven, ascending, brownish to nearly hyaline hyphae about 2-3$\mu$ in diameter with the walls thickened and gelatinized, at the outside becoming thicker and darker forming a rindlike excipulum; asci cylindric, obtuse at the apex, narrowed below to a short stalk, at first

with the walls thickened and gelatinized, becoming thinner with maturity, at first eight-spored, finally multispored, (80)-90-115-(130) × (10)-12-15-(19)μ; primary ascospores hyaline, one- or two-celled, broadly ellipsoid to subglobose, uniseriate, 4.0-5.0 × 2.5-3.5μ; secondary ascospores hyaline, one-celled, cylindric to allantoid, 2.0-3.0 × 1.0-1.5μ; paraphyses hyaline, filiform, septate, simple or branched, 1.5-2.5μ in diameter, the tips slightly swollen and embedded in a brownish, gelatinous matrix forming an epithecium.

Conidial fruiting bodies erumpent, gregarious, separate or cespitose in clusters of up to 20-30, ovoid to subglobose, sometimes more or less laterally compressed, 0.2-0.3 mm. in diameter, the clusters 0.3-0.8 mm. in height, black, glabrous, or sometimes grayish-pruinose, containing a single elongated-ovoid cavity, opening at the top, consistency and tissue structure similar to the apothecia; conidiophores lining the cavity, hyaline, filiform, septate, simple or branched, about 15-60 × 2.0-2.5μ; conidia hyaline, one-celled, cylindric to allantoid, 2.0-3.0 × 1.0-1.5μ, borne at the tip and along the sides of the conidiophore.

*Host:* *Acer* spp.

*Type:* JWG *516*, on *Acer spicatum* Lam., Colchester Co., N.S., Coll. L. E. Wehmeyer, Sept. 7, 1931. LEW *1476*.

*Specimens Examined:*

CANADA: **Quebec:** Gatineau Park, DAOM *13615*, JWG *796*.

UNITED STATES: **New Hampshire:** Pittsburg, JRH *3222*, JWG *844*.

EUROPE: **Hungary:** type of *T. acerina* Rehm, S.

This species was described in a paper by Wehmeyer (1940 (45) ) at which time only the Nova Scotia collection had been seen. Since no specimens of *Tympanis acerina* Rehm had been examined and it was necessary to reject Rehm's name on the grounds of its being a later homonym of *T. acerina* Peck (= *Dermea acerina* (Peck) Rehm), it was decided to describe the Canadian collection as a new species. Through the kindness of Dr. Sten Ahlner, of the Botanical Museum, Stockholm, it has since been possible to examine Rehm's type and it appears to be identical with the North American specimens.

The specimen from the Gatineau Park is the only one which has been studied in the fresh condition and cultured. The description of the conidial fruiting bodies is based on the New Hampshire collection, JWG *844*. In this collection some of the twigs bore black, glabrous, subcespitose pycnidia, whereas on some of the other twigs there were similar pycnidia that were grayish-pruinose and more cespitose. Since no cultures were obtained from this specimen, some doubt might arise as to whether all the twigs bore the same fungus. However, from the range of variation known to occur in other species, such as *T. alnea*, it is probable that these two types of pycnidia are merely variants.

30. TYMPANIS COLUMNARIS v. Höhn. Hedw. 59 : 283. 1918. (Figs. 34, 73)
*Tympanis fraxini* sensu Karst. (non Fr.) Rev. syn. Asc. fenn. 148. 1885.
*T. fraxini* sensu Sacc. Syll. Fung. 8 : 581. 1889.
*T. fraxini* sensu Rehm. Rab. Kr. Fl. I, 3 : 266. 1889.

*T. fraxini* sensu Phill. Man. Brit. Disc. p. 355. 1893.

*T. fraxini* sensu Massee Brit. Fung. Fl. 4 : 130. 1895.

st. conid.

*Sphaeronema columnare* Wallr. Fl. Crypt. Germ. 2 : 763. 1833.

*Pleurophomella columnaris* v. Höhn. Hedw. 59 : 283. 1918.

Apothecia erumpent, scattered to gregarious, separate or sometimes two or three together, sessile, slightly narrowed below, circular to elongated or undulate, 0.5-1.0 mm. in diameter, 0.2-0.5 mm. in height, black, glabrous, hard, horny in consistency, becoming more cartilaginous when moist; hymenium concave to plane, black, becoming paler and greenish when moist, more fleshy than the excipulum, at first with a thick, raised, somewhat inrolled margin which later may disappear; tissue of the hypothecium plectenchymatous, composed of interwoven, ascending, brownish to nearly hyaline hyphae about 2-4$\mu$ in diameter with the walls thickened and gelatinized, at the outside becoming thicker and darker forming a rindlike excipulum; asci cylindric, obtuse at the apex, short-stalked, at first with the walls thickened and gelatinized, becoming thinner with maturity, at first eight-spored, finally multispored, (110)-125-150-(160) $\times$ (13)-14-16-(18)$\mu$; primary ascospores hyaline, ovoid to subglobose, one- or two-celled, uniseriate, 4.0-5.0 $\times$ 3.5-4.5$\mu$; secondary ascospores hyaline, cylindric to allantoid, one-celled, 2.0-4.0 $\times$ 1.0-1.5$\mu$; paraphyses hyaline, filiform, septate, simple or sometimes branched, 1.5-2.0$\mu$ in diameter, the tips slightly swollen and embedded in a brownish gelatinous matrix, forming an epithecium.

*Host:* *Fraxinus* sp.

*Specimens Examined:*

EUROPE: **France,** NYBG; BM in herb. Phillips.

It is difficult to decide what should be regarded as the type of this species. The earliest description that I have seen that certainly refers to the *Tympanis* in question is the one given by Karsten (1885 (21) ). Probably if this specimen is in existence in the Karsten herbarium it should be the type. Rehm (1889 (30) ) cited Fuckel Fung. Rhen. *1125* labeled *Cenangium fraxini* Tul. and, from the account by von Höhnel (1918*b* (19) ), it would appear that he also examined this specimen. However, in the specimen under this number that I was able to examine, I found only a conidial fungus that did not belong either to the *Tympanis* or to the *Dermea*. My concept of this species is based almost entirely on a specimen from France collected by F. Fautrey, No. *2367*, and kindly loaned by Dr. D. P. Rogers from the herbarium of the New York Botanical Garden. Another specimen from the Phillips collections in the herbarium of the British Museum was examined but was not in very good condition. Conidial fruiting bodies were not seen in either of these specimens.

Von Höhnel (1918*b* (19) ) has pointed out that three different Discomycetes and their conidial states occurring on *Fraxinus* have been confused. One of these species is a true *Tympanis* with a *Pleurophomella* as a conidial state, one is a *Dermea* with a *Micropera* as a conidial state, and the third is a *Durandiella* with a *Sphaerographium*-like conidial state. The latter species is *Peziza*

*fraxini* Schw. (= *Tympanis fraxini* (Schw.) Fr.) and is apparently only known from North America. Tulasne (1853 (42) ) described the *Dermea* but misidentified it as *Tympanis fraxini* (Schw.) Fr. and called it *Cenangium fraxini* (Fr.) Tul. Von Höhnel (1915 (16) ) called it *Dermea fraxini* (Tul.) v. Höhn. However, according to the International Rules both the names *Cenangium fraxini* Tul. and *Dermea fraxini* v. Höhn. are obligate synonyms of *Peziza fraxini* Schw. since they are based on the same type. Therefore the *Dermea* was renamed *D. tulasnei* by Groves (1946 (9) ).

The true *Tympanis*, which is known only from Europe, was described by Karsten (1885 (21) ), Rehm (1889 (30) ), Saccardo (1889 (34) ), Phillips (1893 (28) ), and Massee (1895 (22) ) under the name of *Tympanis fraxini* (Schw.) Fr. Von Höhnel (1918*a* (18) ) recognized that *T. fraxini* (Schw.) Fr. was not a true *Tympanis* and placed it in *Godronia* on the basis of the filiform ascospores. It is certainly not, however, congeneric with the type of *Godronia*. Von Höhnel was of the opinion that *Sphaeronaema columnare* Wallr. was the conidial state of the true *Tympanis* and to judge from the description by Wallroth (1833 (44) ), this may well be so. Von Höhnel based this opinion on a statement by Fries that *S. columnare* was identical with *T. fraxini*. He reasoned that Fries must have confused the two fungi since *S. columnare* was found in Germany and *T. fraxini* (Schw.) Fr. only occurs in North America, and concluded that *S. columnare* must have been associated with the true *Tympanis*. Accordingly he named the discomycete *Tympanis columnaris* (Wallr.) v. Höhn. and described the conidial state as *Pleurophomella columnaris* v. Höhn.

This treatment is not in accordance with the International Rules, because Wallroth's fungus was evidently a conidial form and should have been called *Pleurophomella columnaris* (Wallr.) v. Höhn. n. comb. On the other hand, the name for the perfect state should not have been written as a new combination but as a new name.

31. TYMPANIS MUTATA (Fckl.) Rehm. Rab. Krypt.-Fl. I, 3 : 270. 1889. (Figs. 19, 61)

*Cenangium mutatum* Fuckel, Symb. Mycol. p. 270. 1870.

Apothecia erumpent, gregarious, sometimes single, usually cespitose with up to 15 in a cluster, sessile, narrowed below, circular or undulate, 0.4-0.8-(1.0) mm. in diameter, 0.2-0.5 mm. in height, black, glabrous, hard, horny in consistency, becoming more cartilaginous when moist; hymenium concave to plane or sometimes convex, black, more fleshy than the excipulum, at first with a thick, raised margin which later may disappear; tissue of the hypothecium plectenchymatous composed of interwoven, ascending, brownish to nearly hyaline hyphae about 2.0-4.0$\mu$ in diameter with the walls thickened and gelatinized, at the outside becoming thicker and darker forming a rindlike excipulum; asci cylindric, obtuse at the apex, narrowed below to a short stalk, at first with the walls thickened and gelatinized, becoming thinner with maturity, at first eight-spored, finally multispored, (80)-85-110 $\times$ 13-16$\mu$; primary ascospores hyaline, one-celled, ellipsoid to subglobose, uniseriate to

sub-biseriate, about 4.0-5.0 × 3.0-4.0μ; secondary ascospores hyaline, one-celled, cylindric to allantoid, 2.0-3.0 × 1.0-1.5μ; paraphyses hyaline, filiform, septate, simple or branched, 1.5-2.0μ in diameter, the tips slightly swollen and embedded in a brownish, gelatinous matrix, forming an epithecium.

Conidial fruiting bodies usually occurring on the same stroma as the apothecia, ovoid to conic, sometimes more or less collapsed, black, glabrous, 0.2-0.4 mm. in diameter, consistency and tissue structure similar to the apothecia; containing an ovoid cavity, opening at the top; conidiophores lining the cavity, hyaline, filiform, septate, simple, not observed branching, 10-70 × 2.0-2.5μ; conidia hyaline, one-celled, cylindric to allantoid, 2.0-3.0 × 1.0-1.5μ, borne at the tip and along the sides of the conidiophore.

*Host:* *Betula* spp.

*Specimens Examined:*

EUROPE: **Sweden:** Ostrogothia, S.

Fuckel (1870 (7) ) described *Cenangium mutatum* occurring on *Betula*, of which the description suggests a *Tympanis*, but he did not give any ascus measurements and did not cite any specimens. He did state that the asci were multispored as in *T. pithya*. Rehm (1889 (30) ) adopted Fuckel's name for a Swedish collection of a *Tympanis* on *Betula* and transferred it to *Tympanis* although he had not seen any of Fuckel's material. Through the kindness of Dr. Sten Ahlner, Botanical Museum, Stockholm, it has been possible to examine this specimen and the above description is based on it. It is quite distinct from the form of *T. alnea* that may occasionally occur on *Betula*.

A scanty collection of *Pleurophomella*-like fruiting bodies on a twig of *Betula* from New Brunswick was received from Dr. A. J. Skolko. Cultures from these conidia were typically *Tympanis*-like and seemed different from cultures of other *Tympanis* species. However, they have not produced any apothecia in culture as yet, and no apothecia on *Betula* have yet been found. It seems certain that a species of *Tympanis* does occur on *Betula* in North America and it may belong to this species, but this cannot be decided with certainty until the apothecia are found.

32. TYMPANIS SPERMATIOSPORA (Nyl.) Nyl., Notis. Sällsk. Faun. Fl. Fenn. 10 : 70. 1868. (Figs. 38, 57)

*Patellaria spermatiospora* Nyl. Notis. Sällsk. Faun. Fl. Fenn. 1 : 125. 1858.

*Cenangium spermatiosporum* Sacc. Syll. Fung. 8 : 560. 1889.

*Cenangium populinum* Fckl. Symb. Myc. p. 268. 1870.

*Tympanis populina* Sacc. Bot. Centralbl. 18 : 247. 1884.

st. conid.

*Tympanis populi* Lib. nom. nud. Grev. 8 : 85. 1880.

*Pleurophomella spermatiospora* v. Höhn. Fragm. z. Myk. No. 969. 1916.

Apothecia erumpent, gregarious, separate or cespitose in clusters of about two to eight, sessile, slightly narrowed below, circular to undulate, 0.5-0.8-(1.0) mm. in diameter, 0.3-0.5 mm. in height, black, glabrous, hard, horny in consistency, becoming more cartilaginous when moist; hymenium concave to

plane or sometimes convex, black or greenish when moist, more fleshy than the excipulum, at first with a thick, raised margin which later may disappear; tissue of the hypothecium plectenchymatous, composed of ascending, interwoven, brownish to nearly hyaline hyphae about 2-4$\mu$ in diameter, with the walls thickened and gelatinized, at the outside becoming thicker and darker forming a rindlike excipulum; asci cylindric, obtuse at the apex, short-stalked, at first with the walls thickened and gelatinized, becoming thinner with maturity, at first eight-spored, finally multispored, (55)-70-90-(100) $\times$ (9)-10-13-(15)$\mu$; primary ascospores hyaline, one- or two-celled, broadly ellipsoid to subglobose, uniseriate, 5-8 $\times$ 3-4$\mu$; secondary ascospores, hyaline, one-celled, cylindric to allantoid, 2.0-4.0 $\times$ 1.0-1.5$\mu$; paraphyses hyaline, filiform, septate, simple or branched, 1.5-2.0$\mu$ in diameter, the tips slightly swollen and embedded in a brownish, gelatinous matrix, forming an epithecium.

Conidial fruiting bodies erumpent, gregarious, cespitose in clusters of up to 15, occasionally single, ovoid to subglobose or sometimes conical, black, glabrous, 0.2-0.4 mm. in diameter, 0.4-1.0 mm. in height, similar in consistency and tissue structure to the apothecia, containing a more or less ovoid cavity, opening at the top; conidiophores lining the cavity, hyaline, filiform, septate, simple or branched, 15-50 $\times$ 1.5-2.0$\mu$; conidia hyaline, one-celled, cylindric to allantoid, 2.0-5.0 $\times$ 1.0-1.5$\mu$, borne at the tip and along the sides of the conidiophore.

*Host:* *Populus* spp.

*Exsiccati:* Rab.-Kl. Herb. Viv. Myc. *636* (as *Cenangium populi* Lasch); Karst. Fung. Fenn. *752*; Roum. Fung. Sel. Gall. Exs. *654* (as *Cenangium populinum*); Fckl. Fung. Rhen. *766* (as *Tympanis saligna* on *Salix*); Fung. Exs. Suec. praes. Upsal. *1176*; Syd. Myc. March. *2153* (as *T. saligna* on *Salix*).

*Specimens Examined:*

CANADA: **Nova Scotia:** Colchester Co., JWG *800*, LEW *1748*. **Quebec:** Wakefield, DAOM. **Ontario:** Ottawa, DAOM, John Macoun *123*; Forester's Falls, DAOM *3315*, JWG 123; Petawawa Forest Exp. Stn., DAOM *4723*; DAOM *7989*, JWG *771*; DAOM *19882*, JWG *866*; Timagami Forest Reserve, JWG *34*; TRT *2467*, JWG *154*; TRT *6595*, JWG *223*; TRT *7397*, JWG *50*; DAOM *2536*, JRH *2223*. **Manitoba**: Waugh, DAOM. **British Columbia:** Revelstoke, JRH *543*.

UNITED STATES: **Maine:** Indian Township, JRH *2224*; (as *T. morsei* B. & C.) K; Westbrook, FH. **New Hampshire**: Carroll, JRH *1521*, JWG *382*; Jaffrey, FH; Shelburne, FH; Thornton, JRH *1522*, JWG *383*. **Vermont:** Pawlet, FH. **Connecticut:** East Granby, JRH *544*, JWG 336. **Idaho:** DAOM *12058*, UIFP *1745A*, JWG *750*.

EUROPE: **Austria:** Nassau, ex herb. Barbey-Boissier *1102*. **Germany:** Grunenvald, JRH *1205*, S (as *T. saligna* on *Salix*).

*T. spermatiospora* is one of the more common species of *Tympanis*. It was well described by Nylander (1868 (24) ) who observed the primary ascospores. Von Höhnel (1918*a* (18) ) described elongate-fusiform primary ascospores 18-40 $\times$ 3-5$\mu$, but he obviously had a different fungus, possibly *Corynella*

*atrovirens* (Pers.) Boud. The asci were said to be 112-136 × 14-16$\mu$ which is very much too large for *T. spermatiospora*.

Von Höhnel (1916 (17) ) did correctly describe the conidial state, which he named *Pleurophomella spermatiospora*. Sydow and Petrak (1922 (39) ) described the conidial state in considerable detail.

A specimen in Kew Herbarium ex Herbier du Jardin Botanique de Bruxelles and labeled *Tympanis populi* Lib. in Herb. proved to be the conidial state, *Pleurophomella spermatiospora*.

Three European specimens all stated to be on *Salix* agreed morphologically with *T. spermatiospora*. The wood of one of these specimens was checked by Dr. C. G. Riley and found to be *Salix*. It would appear that it may occasionally occur on *Salix* although no collection from this host has been cultured.

Cultures of this species are fairly distinctive, growing rapidly and producing more aerial mycelium than most *Tympanis* species. They also have a characteristic musty odor when freshly isolated.

33. **Tympanis sorbi** sp. nov. (Figs. 29, 58)

Apotheciis erumpentibus, gregariis vel dispersis, solitariis vel subcaespitosis, orbicularibus vel undulatis, sessilibus, versus basim attenuatis, 0.6-1.0 mm. diam., 0.5-0.8 mm. altis, atris, glabris, duris, corneis in sicco, cartilagineis in humido, hymenio concavo, plano vel leviter convexo, atro, carnoso in humido; hypothecio plectenchymato; ascis cylindraceis, breve stipitatis, primo octosporis dein multisporis, (75)-90-115 × (8.5)-11-13$\mu$; ascosporis primariis hyalinis, ellipsoideis vel subglobosis, continuis vel uniseptatis, uniseriatis, 4.0-5.5 × 3.5-4.5$\mu$; ascosporis secundariis hyalinis, unicellularis, cylindricis vel allantoideis, 2.0-4.0 × 1.0-1.5$\mu$; paraphysibus hyalinis, filiformibus, septatis, simplicibus vel ramosis, 2.0-2.5$\mu$ diam., ad apicem leviter incrassatis agglutinatisque, epithecium fuscum formantibus.

Hab.: *Sorbus americana* Marsh.

Apothecia erumpent, gregarious to scattered, separate or subcespitose in small clusters, circular or undulate, sessile, narrowed below, 0.6-1.0 mm. in diameter, 0.5-0.8 mm. in height, black, glabrous, hard, horny in consistency, becoming more cartilaginous when moist; hymenium concave to plane or slightly convex, black, more fleshy than the excipulum, at first with a thick, raised margin which later may disappear; tissue of the hypothecium plectenchymatous, composed of interwoven, ascending, brownish to nearly hyaline hyphae about 1.5-3.0$\mu$ in diameter, with the walls thickened and gelatinized, at the outside becoming thicker and darker forming a rindlike excipulum; asci cylindric, obtuse at the apex, narrowed below to a short stalk, at first with the walls thickened and gelatinized, becoming thinner with maturity, at first eight-spored, finally multispored, (75)-90-115 × (8.5)-11-13$\mu$; primary ascospores ellipsoid to subglobose, hyaline, one- or two-celled, uniseriate, 4.0-5.5 × 3.5-4.5$\mu$; secondary ascospores hyaline, one-celled, cylindric to allantoid, 2.0-4.0 × 1.0-1.5$\mu$; paraphyses hyaline, filiform, septate, simple or branched, 2.0-2.5$\mu$ in diameter, the tips slightly swollen and embedded in a brownish, gelatinous matrix, forming an epithecium.

Conidial fruiting bodies erumpent, scattered, cespitose in small clusters, occasionally single, black or slightly grayish-pruinose, more or less ovoid, about 0.2-0.3 mm. in diam. and 0.3-0.5 mm. in height, consistency and tissue structure similar to the apothecia, containing a single globose to ovoid cavity, tearing open at the top; conidiophores lining the cavity, hyaline, cylindric, septate, unbranched, about 25-70 × 1.5-2.0$\mu$; conidia hyaline, cylindric to allantoid, one-celled, 2.0-4.0 × 1.0-1.5$\mu$; borne at the tip and along the sides of the conidiophore.

*Host:* *Sorbus americana* Marsh.

*Type:* DAOM *26157*, Diamond Lake, Timagami Forest Reserve, Ont., Coll. J. W. Groves, Sept. 2, 1935. Co-type JWG *411*.

This species is known only from the type collection, It can readily be distinguished from *T. conspersa*, also occurring on *Sorbus*, by the large, black apothecia, mostly occurring singly or in small clusters and also by the much smaller asci. This specimen was cultured and the cultures were also different from those of *T. conspersa*. Unfortunately the original material was very scanty and it has not been collected again.

34. **Tympanis malicola** sp. nov. (Figs. 16, 72)

Apotheciis erumpentibus, gregariis vel dispersis, solitariis vel subcaespitosis, 3-4 congregatis, orbicularibus vel undulatis, sessilibus, versus basim attenuatis, 0.5-1.0 mm. diam., 0.3-0.5 mm. altis, atris, glabris, duris, corneis in sicco, cartilagineis in humido; hymenio concavo, plano vel leviter convexo, atro, carnoso in humido; hypothecio plectenchymato; ascis cylindraceis, breve stipitatis, primo octosporis, dein multisporis, 70-85-(100) × 10.0-12.0-(13.0)$\mu$; ascosporis primariis hyalinis, ellipsoideis vel subglobosis, continuis vel uniseptatis, 4.0-7.0 × 3.0-4.0$\mu$; ascosporis secundariis hyalinis, unicellularis, cylindricis vel allantoideis, 2.0-3.0 × 1.0-1.5$\mu$; paraphysibus hyalinis, filiformibus, septatis, simplicibus vel ramosis, 2.0-2.5$\mu$ diam., ad apicem leviter incrassatis agglutinatisque, epithecium fuscum formantibus.

Hab.: *Pyrus malus* L.

Apothecia erumpent, gregarious to scattered, mostly separate, sometimes subcespitose with three or four in a cluster, sessile, narrowed below, circular to undulate, 0.5-1.0 mm. in diameter, 0.3-0.5 mm. in height, black, glabrous, hard, horny in consistency, becoming more cartilaginous when moist; hymenium concave to plane or slightly convex, black, more fleshy than the excipulum, at first with a slightly raised margin which later may disappear; tissue of the hypothecium plectenchymatous composed of interwoven, ascending, brownish to nearly hyaline hyphae about 2.0-3.0$\mu$ in diameter, with the walls thickened and gelatinized, at the outside becoming thicker and darker forming a rindlike excipulum; asci cylindric, obtuse at the apex, narrowed below to a short stalk, at first with the walls thickened and gelatinized, becoming thinner with maturity, at first eight-spored, becoming multispored, 70-85-(100) × (9.0)-10.0-12.0-(13.0)$\mu$; primary ascospores hyaline, one- or two-celled, ellipsoid to subglobose, sometimes slightly fusoid, uniseriate to

partly biseriate, 4.0-7.0 × 3.0-4.0μ; secondary ascospores hyaline, cylindric to allantoid, one-celled, 2.0-3.0 × 1.0-1.5μ; paraphyses hyaline, filiform, septate, simple or branched, 2.0-2.5μ in diameter, the tips slightly swollen and embedded in a brownish, gelatinous matrix, forming an epithecium.

Conidial fruiting bodies erumpent, separate or cespitose with up to 10 in a cluster, black, glabrous, subglobose to ovoid, 0.2-0.4 mm. in diameter, 0.2-0.5 mm. in height, consistency and tissue structure similar to the apothecia, containing a single, more or less ovoid cavity, opening at the top; conidiophores lining the cavity, hyaline, filiform, septate, simple or sometimes branched, 15-50 × 2.0-2.5μ; conidia hyaline, one-celled, cylindric to allantoid, 2.0-4.0 × 1.0-1.5μ, borne at the tip and along the sides of the conidiophore.

*Host:* *Pyrus malus* L.

*Type:* TRT unnumbered. On *Pyrus malus* L., Canton, Mass. Coll. D. H. Linder, Feb. 21, 1933. Ex herb. D. H. Linder.

*Specimens Examined:*

UNITED STATES: **Connecticut:** East Granby, JRH *1138*, JWG *363*.

This species is strikingly distinct from *T. conspersa*, which also occurs on *Pyrus*, in the small asci, scattered habit of growth, and glabrous apothecia. Only the two specimens cited above have been seen and the species has not been studied in culture.

It is close to *Tympanis sorbi*, but the asci are smaller and the primary ascospores more elongated, and the apothecia are less strongly erumpent. It requires further study from fresh material.

35. **Tympanis rehmiana** nom. nov. (Fig. 74)

*Tympanis truncatula* sensu Rehm. Rab. Krypt.-Fl. I, 3 : 277. 1889. (Not *Peziza truncatula* Pers. ex. Fr. Syst. Myc. 2 : 177. 1822.)

Apothecia erumpent, gregarious, separate or cespitose in small clusters, circular or undulate, sessile, narrowed below, 0.5-0.8 mm. in diameter, about 0.5 mm. in height, black, glabrous, hard, horny in consistency, becoming more cartilaginous when moist; hymenium concave to plane, black, more fleshy than the excipulum, at first with a slightly raised margin which later may disappear; tissue of the hypothecium plectenchymatous composed of interwoven, ascending, brownish hyphae about 2-3μ in diameter with the walls thickened and gelatinized, at the outside forming a rindlike excipulum of dark, thick-walled cells about 5-6μ in diameter; asci cylindric, obtuse at the apex, narrowed below into a short stalk, with the walls thickened and gelatinized at first, becoming thinner with maturity, at first four- to eight-spored, finally multispored, (80)-85-100 × (9.5)-11-13-(14)μ; primary ascospores hyaline, fusoid-clavate, sometimes attenuated below, 12-15 × 3-4μ; secondary ascospores hyaline, one-celled, cylindric to allantoid, 2.0-4.0 × 1.0-1.5μ; paraphyses hyaline, filiform, septate, simple or branched, 1.5-2.0μ in diameter, the tips slightly swollen and embedded in a brownish, gelatinous matrix forming an epithecium.

*Host:* *Fagus* sp.

*Type:* Auf abgefallenen Buchenzweigen, Wurttemberg, leg. v. Tavel. 1889. ZT.

This fungus is known only from the collection on *Fagus* cited above. Rehm (1889 (30) ) referred it doubtfully to *Peziza truncatula* Pers. because this was reported on *Fagus* but, as has been shown previously in this paper, the host of Persoon's type was misidentified, and his fungus actually occurred on *Abies*. The cultural studies reported by Brefeld (1891 (3) ) under the name of *Tympanis truncatula* were apparently based on isolations from this same collection.

It is distinctive by reason of the somewhat clavate primary ascospores. These were observed in the material preserved at Zurich and were exactly as described and figured by Brefeld (1891 (3) ).

Since it has now been established that *Peziza truncatula* Pers. is a different fungus, it is necessary to give this species a new name.

36. TYMPANIS CEPHALANTHI Dearn. & House, Bull. N. Y. St. Mus. 266: 63. 1925. (Fig. 33).

st. conid.

*Dendrophoma cephalanthi* Peck. Ann. Rep. N.Y. St. Mus. 39 : 45. 1887.

"Apothecia black, in clusters of two to four, arising from a thin stroma in the basal stratum of the cortex of the host, cespitosely erumpent through the cuticle, nearly plane, 0.4 mm. wide, 0.35 mm. high, with or in a group of somewhat similar pycnidia. Asci very regularly truncate-clavate, 45-60$\mu$ long, 8$\mu$ across the flat tip, 10-12$\mu$ thick at the broadest part, the upper half crowded with minute sporidia, ascus pore not staining blue with iodine, paraphysate. Sporidia very numerous, minute, hyaline, allantoid, 3-4 $\times$ 0.5-1.0$\mu$."

Conidial fruiting bodies erumpent, scattered to gregarious, separate or cespitose, black, glabrous, subglobose to ovoid or somewhat irregular, 0.1-0.5 mm. in diam., containing a single, ovoid to somewhat elongated cavity, opening at the top, hard, horny in consistency, becoming more cartilaginous-leathery when moist; tissue plectenchymatous composed of brownish to nearly hyaline, interwoven hyphae about 2.0-4.0$\mu$ in diameter with the walls thickened and gelatinized; conidiophores lining the cavity, hyaline, filiform, septate, simple or sometimes branched, about 10-50 $\times$ 2.0-2.5$\mu$; conidia hyaline, one-celled, cylindric to allantoid, 2.0-4.0 $\times$ 1.0-1.5$\mu$, borne at the tip and along the sides of the conidiophore.

*Host:* *Cephalanthus occidentalis* L.

*Specimens Examined:* NYBG. in Rel. Farl. *103*; ex herb. John Dearness, coll. Peck. July.

This species was described by Dearness and House (l.c.) based on apothecia which they found associated with *Dendrophoma cephalanthi* Peck, and which they took to be the perfect stage. In a specimen kindly loaned by Dr. Dearness, I was unable to find any apothecia but only the fruiting bodies of a

*Pleurophomella*. In another specimen, loaned by Dr. D. P. Rogers from the New York Botanical Garden, of Reliquiae Farlowianae *103* labeled *Cenangium cephalanthi* Fr., there were also some *Pleurophomella* fruiting bodies and one immature apothecium. Most of the material in this specimen was actually *Cenangium cephalanthi* Fr., a different fungus.

Since I have seen no satisfactory material of apothecia of this species I have quoted the original description. The description of the asci would suggest that the material was immature or abnormal and the species requires restudying from fresh material. However, it is evident from the material examined that a species of *Tympanis* does occur on *Cephalanthus*, hence it has been included, even although inadequately treated.

## Generic Host Index

*Abies*
*T. abietina*
*T. truncatula*

*Acer*
*T. acericola*

*Alnus*
*T. alnea*
*T. alnea* var. *hysterioides*

*Amelanchier*
*T. amelanchieris*

*Betula*
*T. alnea*
*T. mutata*

*Cephalanthus*
*T. cephalanthi*

*Diospyros*
*T. diospyri*

*Fagus*
*T. rehmiana*

*Forsythia*
*T. forsythiae*

*Fraxinus*
*T. columnaris*

*Hydrangea*
*T. hydrangeae*

*Juniperus*
*T. juniperina*

*Larix*
*T. laricina*

*Ligustrum*
*T. ligustri*

*Magnolia*
*T. magnoliae*

*Myricaria*
*T. myricariae*

*Oxydendrum*
*T. oxydendri*

*Picea*
*T. piceae*
*T. piceina*

*Pinus*
*T. confusa*
*T. pithya*
*T. hypopodia*

*Populus*
*T. spermatiospora*

*Prunus*
*T. conspersa*
*T. prunicola*

*Pseudotsuga*
*T. hansbroughiana*
*T. pseudotsugae*

*Pyrus*
*T. conspersa*
*T. malicola*

## Generic Host Index—*Concluded*

*Rhus*
*T. rhoina*

*Salix*
*T. salicina*
*T. saligna*
*T. spermatiospora*

*Sorbus*
*T. conspersa*
*T. sorbi*

*Syringa*
*T. syringae*

*Tsuga*
*T. tsugae*

*Viburnum*
*T. fasciculata*

## Doubtful and Excluded Species

*Tympanis abietis* Crouan, Fl. d. Finist. p. 43, 1867. No material of this species has been seen and the description is very incomplete. It is said to have multispored asci but has been excluded from *Tympanis* on the basis of the globose, secondary ascospores. It might be *Retinocyclus olivaceus* Fckl., Symb. Myc. Nachtr. 1 : 332. 1871.

*Tympanis acerina* Peck, Ann. Rep. N.Y. St. Mus. 31 : 48. 1879 = *Dermea acerina* (Pk.) Rehm, Ber. Bayer. Bot. Ges. 13 : 197. 1912.

*Tympanis acicola* Fckl., Fung. Rhen. *1965*. 1867. The name and description were published on the label of this specimen. There is no evidence that it is intended to be the same fungus as *Cenangium acicolum* (Fckl.) Rehm, Rabh. Krypt.-Fl. I, 3, 228. 1889 (= *C. ferruginosum* var. *acicolum* Fckl., Symb. Myc. p. 269. 1870). The asci were said to be eight-spored and the spores 8 × 2$\mu$. The specimen of Fung. Rhen. *1965* in the Farlow Herbarium was examined but it was very scanty and no mount was made. It was obviously not a *Tympanis*.

*Tympanis amphibola* (Massal.) Karst., Symb. Myc. Fenn. p. 252. 1871 = *Pragmopora amphibola* Massal., Framm. Lich. p. 13. 1855. This fungus has the aspect of a *Tympanis* but the ascospores are fusiform, 14-20 × 3-4$\mu$, and the asci do not become multispored.

*Tympanis amphiboloides* Nyl., Obs. Pez. Fenn. p. 71. 1868. In his description of the species, said to occur on *Pinus sylvestris*, Nylander described only eight-spored asci with spores 18-23 × 2.5-3.5$\mu$. He only described multispored asci in his account of the variety *hypopodiza* which is here considered to be a synonym of *T. confusa* Nyl. The description of *T. amphiboloides* suggests a *Pragmopora* and, although Nylander states that he compared it with *P. amphibola* Massal. and thought it different, there is little in the description to separate the two. Unfortunately no authentic material has been seen but it is considered to be probably a *Pragmopora*. Rehm (1889 (30)) reported it as occurring on *Quercus*, but a specimen so labeled on this host in the herbarium at Zurich proved to be *Corynella atrovirens* (Pers.) Boud.

*Tympanis antarctica* Speg., Bolet. Acad. Nac. Cienc. de Cordoba. 11 : 270. 1887. No material of this species has been seen, but the description suggests that it may be a true *Tympanis* species. The original description is quoted as follows: "Cupulae dense caespitosae, per rimas lineares transversas peridermii erumpentes, 10-50 in quoque caespitulo, minutae (250-350$\mu$ diam.) primo sphaeroideo-clausae dein irregulariter apertae, extus atrae subcarbonaceae glabrae, intus vix pallescentes; asci cylindraceo-clavati, apice obtuse lateque rotundati, deorsum brevissime crasseque noduloso-stipitati (75-80 $\times$ 10-14$\mu$), myriospori, paraphysibus filiformibus longioribus dense obvallati; iodi ope nulla; sporidia inordinata conglobata muco immersa minuta cylindracea recta v. allantoidea (2-2.5 $\times$ 0.7-1$\mu$) utrinque plus minusve rotundata hyalina. Hab. Ad ramos emortuos *Fagi betuloidis* in sylvis prope Agaia, Jun. 1882."

*Tympanis aparines* Wallr., Fl. Crypt. Germ. 2 : 424. 1833. This may be the same fungus as *Pyrenopeziza galii-veri* (Karst.) Sacc., Syll. Fung. 8 : 356. 1889. It is not a *Tympanis*.

*Tympanis ariae* Fr., Syst. Myc. 2 : 175. 1822 = *Dermea ariae* (Pers. ex Fr.) Tul. ex Karst., Myc. Fenn. 1 : 224. 1871.

*Tympanis atra* (Rabenh.) Karst., Symb. I, p. 25, 1871. Rabenhorst (1844, p. 342) described a fungus under the name *Lecanidion atrum* and cited *Patellaria atrata* Fr. as a synonym. There is no apparent reason why he changed the specific epithet. A specimen in Rabh. Fung. Eur. *33* was issued under this name and this fungus is undoubtedly a *Tympanis*, but the specimen I examined was wholly immature and could not be identified. Karsten (l.c.) made the combination *T. atra* based on this specimen, but since it was a misidentification of *Patellaria atrata* Fr., the name *T. atra* (Rabh.) Karst. must be a synonym of *P. atrata* and the name is invalid for any species of *Tympanis*.

*Tympanis atrovirens* (Pers.) Rehm, Hedw. 21 : 70. 1882 = *Corynella atrovirens* (Pers.) Boud., Disc. d'Eur. p. 99. 1907. This fungus is very like a *Tympanis* in gross appearance and in the fact that the asci become multispored. It is excluded from the genus because of its more superficial growth habit, usually occurring on somewhat weathered wood, its lack of host specificity, and somewhat softer consistency than typical *Tympanis* species.

*Tympanis bacillifera* Karst., Symb. Myc. Fenn. 1 : 252. 1871 = *Pragmopora bacillifera* (Karst.) Rehm, Rab. Krypt.-Fl. I, 3 : 340. 1890. This fungus is obviously closely related to *P. amphibola* Massal. from which it can be distinguished by the longer ascospores.

*Tympanis bicolor* Ellis, Amer. Nat. 17 : 193. 1883 = *Dermea bicolor* (Ell.) Groves, Mycologia 35 : 460. 1943.

*Tympanis buchsii* (Henn.) Rehm, Ber. d. Bayer Bot. Ges. 13 : 205. 1912. This was described by Hennings (1903 (13) ) on *Abies* as the type of a new genus *Biatorellina*. The asci were said to be 70-100 $\times$ 10-18$\mu$ and multispored. Rehm (l.c.) found eight spores in the asci that were elliptic, hyaline,

18 × 10μ. He thought they were primary ascospores and that *Biatorellina* should be a synonym of *Tympanis*. Von Höhnel (1918*a* (18) ) claimed that the type was immature *Tryblidiopsis pinastri* (Pers. ex. Fr.) Karst. I have not seen any material.

*Tympanis bupleuri* Vel., Monogr. Disc. Bohem. p. 67. 1934. This fungus was described as occurring on the stems of many different herbaceous plants. The description suggests that it is close to *Tympanis*, but the wide host range and occurrence on herbaceous plants make it very doubtful that it is a true *Tympanis*. No material has been seen.

*Tympanis cerasi* Quel., Enchir. Fung. p. 330. 1886 = *Dermea cerasi* (Pers ex Fr.) Fr., Syst. Orb. Veg. p. 115. 1825.

*Tympanis cinerascens* Schw., Syn. Fung. Amer. bor. p. 237. 1832. A portion of the type in the Kew Herbarium was examined. It appeared to be on a conifer and suggested immature *Tryblidiopsis pinastri* (Pers. ex Fr.) Karst. in appearance. Sections revealed a pseudoparenchymatous structure that definitely eliminated *Tympanis*. It could not be identified with certainty. A specimen at Uppsala labeled "*Tympanis cinerescens* Schw. Herb. E. Fries" was not a discomycete. It suggested an immature *Hypoxylon* and a mount yielded dark brown, two-celled conidia 13-17 × 7-9μ.

*Tympanis corylina* (Sacc.) Rehm, Rab. Krypt. Fl. I, 3 : 270. 1889. In the Syll. Fung. 8 : 583. 1889, Saccardo accepted Rehm's name for this fungus which he had originally published as *Chilangium*. The asci were said to be multispored and the spores 6-8 × 1μ, which is too long for any known *Tympanis*. No Saccardo specimens have been seen but, through the kindness of Dr. Sten Ahlner, three specimens from the Rehm Herbarium in the Botanical Museum, Stockholm, have been examined. One of these was *Pezicula ocellata* (Pers.) Seav. and was not on *Corylus*, and the other two were both *Catinula turgida* (Fr.) Desm., the conidial state of *Pezicula corylina* Groves.

*Tympanis crataegi* Lasch, Bot. Zeitg. 19 : 295. 1861. This species was based on the specimen in Rabh. Fung. Eur. *353* which is a *Pezicula*.

*Tympanis cupularis* Wallr., Flor. Crypt. Germ. 2 : 428. 1833 = ? *Nitschkia cupularis* (Pers. ex Fr.) Karst., Myc. Fenn. 2 : 81. 1873. European authors have cited Wallroth's name as a synonym of *N. cupularis* and Fitzpatrick (1923 (5) ) has pointed out that *N. cupularis* is a composite species and the concept of the species is confused. Four specimens labeled *T. cupularis* Wallr. in Kew Herbarium were examined. They were not in good condition but were evidently a pyrenomycete.

*Tympanis farinacea* (Pers.) Rehm, Rab. Krypt.-Fl. I, 3 : 277. 1889 = *Cenangium ferruginosum* Fr., Syst. Myc. 2 : 187. 1822. A specimen in the Persoon Herbarium at Leiden No. *910.261.960* is undoubtedly to be interpreted as the type and is *Cenangium ferruginosum*. Also a specimen in the herbarium at Uppsala labeled Fries Sclerom. *294*, *Peziza farinacea* b. Syst. Myc., looked like *C. ferruginosum*, but in three mounts examined the fungus was immature and there were no asci or spores.

*Tympanis frangulae* (Pers.) Fr., Syst. Myc. 2 : 174. 1822 = *Pezicula frangulae* (Pers. ex Fr.) Fckl., Symb. Myc. p. 279. 1870.

*Tympanis fraxini* (Schw.) Fr., Syst. Myc. 2 : 174. 1822 = *Durandiella fraxini* (Schw. ex. Fr.) Seaver, N. Amer. Cup. Fungi. Inoperc. p. 367. 1951.

*Tympanis fuliginosa* (Pers. ex. Fr.) Wallr., Flor. crypt. germ. 2 : 429. 1833 = *Scleroderris fuliginosa* (Pers. ex. Fr.) Karst., Myc. Fenn. 1 : 216. 1871.

*Tympanis galii* Rabenh. This name is cited by Oudemans, Enum. Syst. Fungi. 4 : pp. 759 and 768. 1923 as a synonym of *Cenangium aparines* (Wallr.) Fuckel and *Ephelina galii* (Lasch) Sacc. respectively. Reference was made to Rabenh. Krypt. Fl. but I was unable to find the name here or trace it elsewhere. It is doubtful that Rabenhorst made the combination.

*Tympanis gyrosa* B. & C., Grev. 4 : 3. 1875. The original description was very brief and incomplete. Two specimens were cited, No. *3338* and No. *5940*. In the Kew Herbarium there are two specimens, one bearing the number *3538*, Virg. Mts. and the other number *940* New England, Russell. These are obviously the specimens cited in Grevillea as *3338* and *5940*.

Under the Russell number there is no specimen present. Under No. 3538 (3338 ?) there were a few small, black, somewhat elongated apothecia. In a mount I found some fragments of asci and one intact ascus 77.0 × 13.0$\mu$, eight-spored. The spores were 12-15 × 7-8.5$\mu$, ellipsoid, brownish, and three-septate. I could not identify it but it is certainly not a *Tympanis*.

*Tympanis inconstans* Fr., Summ. Veg. Scand. p. 400. 1849 = *Dermea ariae* (Pers. ex. Fr.) Tul. ex. Karst., Myc. Fenn. 1 : 224. 1871.

*Tympanis kmetiana* D. Sacc. in Contrib. alle fl. mic. di Schemnitz p. 21. 1897. Through the kindness of Dr. Sten Ahlner, a specimen from the Botanical Museum, Stockholm, has been examined. The specimen was collected by A. Kmet and appears to be part of the type. The fungus has the aspect of a small, grayish-pruinose *Encoelia*. The asci are eight-spored, 36-48 × 4-5$\mu$ and the ascospores are hyaline, ellipsoid-fusiform, one-celled, about 4-5 × 1.5-2.0$\mu$. The spores were not observed free in the mount examined, and hence may be immature. The fungus cannot be placed satisfactorily but is certainly not a *Tympanis*.

*Tympanis lonicerae* Fr., Elench. Fung. 2 : 19. 1828. A specimen ex. herb. Fries in the herbarium at Uppsala was the only material examined. This specimen consisted of two twigs with a few black, erumpent stromata but no apothecia could be found.

*Tympanis macrospora* Karst., Rev. Mon. Ascom. Fenn. p. 147. 1885 = *Patellaria macrospora* (Bagl.) Karst. Mycol. Fenn. I : 235. 1871. This species was described as having four- to eight-spored asci with spores 32-36 × 10-15$\mu$. No material has been seen but it is certainly not a *Tympanis*. Rehm, Rab. Krypt. Fl. I, 3 : 335. 1889, considered it to be a synonym of *Patellaria carestiae* De Not.

*Tympanis microtheca* (Karst.) Rehm, Rab. Krypt.-Fl. I, 3 : 275. 1889 = *Tromera microtheca* Karst., Meddel. Soc. Faun. Fl. Fenn. 16 : 22. 1888. Through the kindness of Dr. Sten Ahlner, two specimens from the Rehm Herbarium in the Botanical Museum, Stockholm, were examined. One of these was apparently part of the type and both agreed well with the original description. I could not place the fungus satisfactorily, but it must be excluded from *Tympanis* on the basis of the character of the asci and the consistency and tissue structure of the apothecia.

*Tympanis nemopanthis* Peck, N.Y. St. Mus. Rep. 35 : 142. 1884 = *Durandiella nemopanthis* (Pk.) Groves, Mycol. 29 : 75. 1937.

*Tympanis obtexta* Wallr., Fl. Crypt. Germ. 2 : 428. 1833. Specimens under this name in Karst. Fung. Fenn. *276* appeared to be a *Tapesia* whereas those in Kl. Herb. viv. Myc. *1138* were another fungus. In this latter specimen the asci were 32-50 × 6-8$\mu$, eight-spored with a few free spores 6-10 × 2-3$\mu$. Wallroth's description does not suggest a *Tympanis* and there does not appear to be any clear concept of his species. It should certainly be excluded from *Tympanis*, and may be the same as *Tapesia rosae* (Pers. ex Fr.) Fckl.

*Tympanis oleastri* Pass. & Thüm., Hedw. 21 : 15. 1882 = *Blytridium oleastri* (Pass. & Thüm.) Sacc., Syll. fung. 8 : 806. 1889. No material of this species has been seen but the colored muriform spores 24 × 12-13$\mu$ would certainly exclude it from *Tympanis*.

*Tympanis olivacea* (Fckl.) Rehm. Rab. Kr. Fl. I, 3 : 275. 1889 = *Retinocyclus olivaceus* Fckl., Symb. Myc. Nachtr. 1 : 332. 1871. This species is close to *Tympanis* but has been excluded from the genus chiefly because of its more superficial habit of growth and softer consistency. It occurs on resin and does not appear to be limited to any particular host. It can be easily recognized by the globose secondary ascospores.

*Tympanis padi* Quel., Enchir. Fung. p. 330. 1886 = *Dermea padi* (Alb. & Schw.) Fr., Summ. Veg. Scand. p. 362. 1849.

*Tympanis patella* (Fr.) Wallr., Flor. Crypt. Germ. 2 : 425 1833 = *Heterosphaeria patella* Grev., Scott. Crypt. Fl. 2 : 103. 1824. This fungus, occurring on the stems of Umbelliferae appears to be known under various names but is certainly not a *Tympanis*.

*Tympanis pezizoides* Rab. in Hoffm. Icon. Anal. fung. p. 97, t. 23, f.l. 1865 = *Phaeodiscula pezizoides* (Rabh.) Sacc. & Trott., Syll. Fung. 22 : 1173. 1913. From the Saccardo description this is evidently a conidial fungus.

*Tympanis picastra* Berk. & Curt. nom. nud., Rav. Fung. Carol. No. 63. 1852 = *Tympanis ravenelii* Berk., Grev. 4 : 3. 1875.

*Tympanis pini* Lib. nom. nud., Grev. 8 : 85. 1879. A specimen in Kew Herbarium labeled *Tympanis pini* Lib. in Herb. bore only small black pycnidia with hyaline, one-celled, ellipsoid to ovoid spores, 4-7 × 2-3$\mu$.

*Tympanis pithya* (Fr.) Sacc., Syll. Fung. 8 : 583. 1889 = *Cenangium pithyum* Fries, Syst. Mycol. 2 : 184. 1822. The specimen in Fries Sclerom.

Suec. *172* labeled *Excipula pithya* Fr. has eight-spored asci with filiform, many-septate spores. It is not a *Tympanis*.

*Tympanis plicato-crenata* (Schw.) Fr., Syst. Myc. 2 : 175 1822. A specimen under this name in Kew Herbarium was a fungus belonging in the *Patellariaceae* with eight-spored asci mostly 100-130 × 12-14$\mu$ and ascospores hyaline, fusoid to clavate, 5-8 septate, 25-36 × 5.5-8.0$\mu$. It is not known whether or not this is an authentic specimen but the original description does not suggest a *Tympanis*. The asci and spores were not described.

*Tympanis plinthis* Rab., Kr. Fl. p. 338. 1844 = *Phacidium plinthis* Fr., Elench. Fung. p. 134. 1828. No material of this species has been seen but the description does not suggest a *Tympanis* and its occurrence on *Scirpus* makes it very improbable that it belongs in this genus.

*Tympanis polygonia* (Fckl.) Sacc., Syll. Fung. 8 : 579. 1889 = *Cenangium polygonium* Fckl., Symb. Myc. Nachtr. II : 55. 1873. This species, described on *Pyrus*, was said to have multispored asci 108 × 20$\mu$, suggesting a *Tympanis* and probably *T. conspersa*, but the description of the apothecia did not suggest a *Tympanis*. According to Rehm (1889 (30) ) who was himself unable to find any spores in Fuckel's specimen, Minks had found eight-spored asci with immature ellipsoid spores 15-20 × 6-8$\mu$. Rehm transferred it to *Dermea*. Saccardo (1889 (34) ) concluded that there were two fungi and recognized the eight-spored form as *Cenangium polygonium* Fckl. and made a new combination, *Tympanis polygonia*, supposedly based on the multispored form. Both of these combinations were based on Fuckel's type, but apparently no one except Fuckel has claimed to have found multispored asci. Von Höhnel (1918*a* (18) ) argued that it was an old specimen of *T. piri* (= *T. conspersa*), but he did not see multispored asci and his argument is based on the presence of a conidial fungus with oblong-ellipsoid spores, *Discosporium pyri* v. Höhn. which he believed to be the conidial state of *T. conspersa*. However, we now know that this is not the conidial state of *T. conspersa*, hence von Höhnel's observations tend to strengthen the view that *C. polygonium* is not a *Tympanis*. I have examined a specimen ex herb. Barbey-Boissier *1104* in which the apothecia agree with the description but in which the asci are eight-spored (100)-110-125-(160) × (14)-15-18$\mu$ and the ascospores oblong-ellipsoid to ovoid, hyaline, one-celled, 18-25 × 7.5-10$\mu$. It is closer to *Dermea* than to *Tympanis* but should be studied in culture in order to place it satisfactorily. It seems likely that some apothecia of *T. conspersa* were present in Fuckel's original specimen and that he mounted one of these. The original description is probably compounded from two fungi and it should be regarded as a *nomen confusum*.

*Tympanis potentillae* Hazslinsky. In a *Cenangella* folder in Kew Herbarium there is material labeled *T. potentillae* Hazsl. Nannfeldt, Trans. Brit. Myc. Soc. 20 : 202. 1936, expressed the opinion that this was actually *Dermea cerasi* (Pers. ex. Fr.) Fr. and was on *Prunus* rather than *Potentilla*. The wood was not checked microscopically but, from an examination of the fungus and from the gross appearance of the host, I am in agreement with Nannfeldt.

*Tympanis prunastri* Wallr., Flor. Crypt. Germ. 2 : 427. 1833 = *Dermea prunastri* (Pers. ex Fr.) Fr., Summ. Veg. Scand. p. 362. 1849.

*Tympanis punctoidea* Cke., Bull. Buff. Soc. Nat. Science Part III, p. 30. 1875. Three specimens under this name have been examined, two in the Kew Herbarium and one in the Herbarium of the British Museum. One of the specimens in Kew is marked type, collected at Potsdam, N.Y. The host is not named but the fungus is unquestionably *Dermea acerina* (Pk.) Rehm. The second Kew specimen, collected in Maine also on an unidentified host, is the conidial state of a *Scleroderris*. The specimen in the British Museum is an immature pyrenomycete on *Ribes*. The name should become a synonym of *Dermea acerina* (Pk.) Rehm.

*Tympanis ravenelii* Berk., Grev. 4 : 3. 1875. This fungus was described as occurring on *Carpinus* and *Ilex*. A specimen labeled *Tympanis ravenelii* Berk. & Curt sp. nov. said to be on *Carpinus*, was examined in the Farlow Herbarium. I am of the opinion that the host was misidentified and that this specimen is also on *Ilex*. It is a striking fungus with large, two-celled ascospores and has somewhat the gross appearance of a *Dermea*. Seaver (1951 (38) ) places it in *Cenangella*, but this treatment is untenable since he uses the genus *Cenangella* in a sense which excludes the type.

*Tympanis repanda* (Fr.) Rab., Handb. I : p. 338. 1844 = *Scleroderris ribis* (Fr.) Keissler, Krypt. Exs. Mus. Palat. Vind. *2029*.

*Tympanis ribis* (Fr.) Wallr., Flor. Crypt. Germ. 2 : 430. 1833 = *Scleroderris ribis* (Fr.) Keissl. Krypt. Exs. Mus. Palat. Vind. *2029*.

*Tympanis rosae* Karst., Meddel. Soc. Faun. Fl. Fenn. 16 : 6. 1888. No material of this species has been seen but from the description it may be a true *Tympanis* species. The following description is quoted from Saccardo, Syll. Fung. 8 : 580. 1889. "Cupulis sessilibus vel subsessilibus, solitariis vel caespitulosis, nudis; nigris planiusculis, margine subacuto, subdenticulato-inaequali, nitentibus, 0.4 mm. lat.; ascis cylindraceo-subclavatis, myriosporis, 75 × 9-10$\mu$; sporidiis oblongis vel ellipsoideis, 2 × 0.5-1$\mu$; paraphysibus gracilibus apice conglutinatis.

Hab. in ramis *Rosae volvatae* in horto Mustiala Fenniae."

*Tympanis rosae* (Pers. ex. Fr.) Wint. This combination was cited by Oudemans, Enum. Syst. Fung. 2 : 363. 1920, with a reference to Rab. Kr.-Fl. 3, p. 582, but no such combination could be found here or traced elsewhere. Possibly "*T. rosae*" referring to *Tapesia rosae* was misinterpreted as *Tympanis*.

*Tympanis rubi* (Fr.) Wallr., Flor. Crypt. Germ. 2 : 424. 1833 = *Pyrenopeziza rubi* (Fr.) Rehm, 26 Ber. Naturh. Ver. Augsburg p. 101. 1881.

*Tympanis saepiaria* Karst., Symb. ad Mycol. Fenn. 13 : 22. 1871. Through the kindess of Dr. D. P. Rogers, a Karsten specimen of this species in the herbarium of the New York Botanical Garden was examined. It could not be placed satisfactorily but is certainly not a *Tympanis*. The asci were

eight-spored, 65-90 × 7-9$\mu$ and the spores hyaline, fusoid, one- or two-celled, 12-17 × 3.5-4.0-(5.0)$\mu$.

*Tympanis sambuci* Cr., Fl. de Finist. p. 43. 1867. No material of this species has been seen, but it can be excluded from *Tympanis* because the apothecia are said to be yellowish with eight-spored asci and subfusiform ascospores.

*Tympanis seriata* Schw., Syn. Fung. Amer. bor. p. 237. 1832. No material has been seen. The fungus was said to occur on unidentified, decorticated wood, and the description does not suggest a *Tympanis*.

*Tympanis stictica* B. & C., Grev. 4 : 3. 1875 = *Cenangium sticticum* (B. & C.) Sacc., Syll. Fung. 8 : 559. 1889. The original description was very incomplete, but the spores were said to be "fusiform, slightly curved, with 4 nuclei", which suggests that it was not a true *Tympanis*. A specimen in Kew Herbarium labeled "75, Car. Sup. on Vitis, M. A. Curtis" (presumably the specimen cited as *175* in the original description) was examined, but I could find no asci or spores. It was not a *Tympanis*.

*Tympanis tautziana* Ruhland, Verhandl. Bot. Ver. Brandbg. 41 : 83. 1899 = *Scoleconectria scolecospora* (Bref.) Seav., Mycol. 1 : 198. 1909. Through the kindness of Dr. Sten Ahlner, the type from the Botanical Museum, Stockholm, was examined. It is not a discomycete.

*Tympanis toomansis* B. & Br., Trans. Linn. Soc. Lond. 2nd ser., 2 : 222. 1887. This name was based on an immature Australian fungus on *Banksia*. The type in the British Museum has been examined It is an *Encoelia*-like fungus, about 2 mm. in diameter and 3 mm. in height with a short thick stipe. It cannot be placed satisfactorily in the absence of asci and spores but is certainly not a *Tympanis*.

*Tympanis turbinata* Schw., Syn. Fung. Amer. bor. p. 237. 1832 = *Godronia turbinata* (Schw.) Farl., Mycol. 14 : 101. 1922. For a discussion of this species see the notes on *T. fasciculata* Schw.

*Tympanis turgida* Wallr., Fl. Crypt. Germ. 2 : 424. 1833 = *Catinula turgida* (Fr.) Desm., Ann. Sci. Nat. III, 18 : 374. 1852. This is an imperfect fungus, the conidial state of *Pezicula corylina* Groves.

*Tympanis urceolus* Wallr., Flor. Crypt. Germ. 2 : 425. 1833 = *Cenangium urceolus* Fr., Syst. Mycol. 2 : 182. 1822. The concept of this fungus is very much confused but it might be pointed out that *Cenangium urceolus* Fr. (l.c.) on *Betula*, is not the same fungus as *Peziza urceolus* A. & S. ex Fr. Syst. Myc. 2 : 113. 1822, on *Alnus* and that neither of these is congeneric with the type of the genus *Godronia*. *C. urceolus* Fr. is a *Scleroderris* occurring on *Betula* and is not closely related to *Tympanis*.

*Tympanis vagabunda* Pass. & Belt., Hedw. 22 : 120. 1883 = *Cenangium vagabundum* (P. & B.) Sacc., Syll. Fung. 8 : 557. 1889. No material of this fungus has been seen, but it was described as having eight-spored asci with oblong-ellipsoid spores 12 × 5$\mu$.

*Tympanis vermicularis* Ces., Atti R. Acc. Sci. fisich. e matem. di Napoli 8 : 11. 1879 = *Scleroderris vermicularis* (Ces.) Sacc., Syll. Fung. 8 : 598. 1889. This species is known only from the description in Saccardo (l.c.). It was described from Borneo and the spores were said to be several septate, 24$\mu$ long.

*Tympanis viticola* (Schw.) Fr., Syst. Myc. 2 : 176. 1822. A portion of the type in the Kew Herbarium has been examined as well as specimens loaned from the New York Botanical Garden through the kindness of Dr. D. P. Rogers. Seaver (1945 (37) ) transferred it to *Godronia* erroneously citing Farlow as the author. It has elongate-fusiform spores but is not congeneric with the type of *Godronia*. It probably belongs in *Durandiella*.

*Tympanis xylophila* Kirschst., Ann. Mycol. 36 : 373. 1938. No material has been seen, but there is nothing in the description to distinguish it from *Corynella atrovirens* (Pers.) Boud.

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# PERSISTENCE OF *RHIZOCTONIA SOLANI* KÜHN IN SOIL[1]

By G. B. Sanford[2]

## Abstract

The effect of different living host and nonhost plants 21 days old, soil amendments, and *Trichoderma lignorum* (Tode) Harz on the persistence of a heavy artificial infestation of *Rhizoctonia solani* Kühn in a natural virgin Edmonton black loam of optimum moisture content, held at 61° F., was studied under controlled laboratory conditions. A disease rating of host plants was found to be a satisfactory measure of the persistence of *R. solani*, whereas data from buried glass slides and from root colonization of indicator plants were unreliable for the purpose intended. In natural, unamended soil, a heavy infestation of *T. lignorum* did not check the growth of *R. solani* perceptibly, but with the addition of cornmeal it was practically suppressed for several days. In the presence of living potato plants, *R. solani* persisted as well in natural untreated soil as it did when cornmeal, sodium nitrate, or calcium hydroxide was added. The living or dead roots of potato or wheat plants did not aid or suppress the pathogen. Apparently *R. solani* disappeared from the soil in 120 days or less when associated with wheat, oat, barley, or corn plants replanted every 21 days, but it was still fairly abundant in soils bearing potato, bean, or pea plants. When potato plants were absent, a heavy soil infestation in the late autumn practically disappeared by the following June. Results indicate that *R. solani* depends essentially on parasitic nutrition for its persistence in the soil. The hyphae of *R. solani* commonly inhabited the living roots of host and nonhost plants, but no nutritional relationship with them was detected.

## Introduction

In a previous study (10), it was found that of 18 different supplements added to natural black loam, certain ones, notably sodium nitrate and cornmeal, tended to reduce the persistence of *Rhizoctonia solani* Kühn. Many of the other chemicals, even if applied in relatively large amounts, had little or no effect. The pathogen seemed to persist best on potato plants in natural soil without amendments. The apparent decline of the pathogen in the soil was tentatively attributed to the antibiotic effects of the soil flora as modified by the amendments, rather than to the soil nutrients added. It was also concluded that the effects of soil temperature (61° F.), water content (optimum), and pH value (6.2) of the soil used, were not determining factors. The foregoing conclusions were based on six separate experiments in temperature controlled chambers, wherein the persistence of the pathogen was indicated by lesions on 21-day-old potato stems during from six to eight successive plantings of the host in the same soil. The results from the experiments just mentioned suggested that evidence should be secured on: whether *R. solani* actually declined in the experimental soil despite the presence of a susceptible host; the rate of decline of the inoculum; whether such decline was affected more by one susceptible host than by another; and whether the decline was more delayed as a result of lesions produced by the susceptible hosts than through sustenance from the roots of the different kinds of plants used.

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## Materials and General Methods

The persistence of *R. solani* was indicated by one or more of the following three methods: a comparative rating of disease severity on certain host plants; the rated abundance of the pathogen on the roots of the various crop plants under test; and the rated growth of the pathogen as observed on microscope slides buried in the test soil and examined periodically. *R. solani* is particularly suitable for this study owing to the relatively large diameter of its hyphae, its distinctive morphology, and its inability to produce spores under the conditions of these experiments. Further, this fungus is commonly regarded as being able to persist saprophytically in the soil and also parasitically on the underground stems of a number of different host plants.

The soil used was unsterilized black Edmonton virgin loam, as described in a previous study (10). It was maintained in a temperature control chamber at approximately 61° F., and at approximately optimum moisture content, by the addition of a very small quantity of water at each replanting. The soil was held in 1-liter Erlenmeyer flasks or in 1-gal. crocks, as required. Potato sets of 10 gm. were cut from clean, semidormant tubers of the Warba variety, stored at about 36° F. These were immersed for eight minutes in a 1-1000 solution of mercuric chloride to which was added 1% by volume commercial hydrochloric acid. Immediately after treatment, the sets were rinsed in tap water and planted about five inches deep in the soil, with the 'eye' up.

A virulent culture of *R. solani* (Culture No. 76), which was increased in steam sterilized black loam, was added at the rate of one part of inoculum to nine parts of natural soil. All the infested soil was thoroughly mixed at one time in one pile and then added to the containers as required.

## Experimental Results

*Experiment 1*

*Effect of Cornmeal in Soil on Persistence of R. solani*

In a previous study (10) unsterilized cornmeal, when added to the experimental soil, reduced the severity of the lesions on potato stems caused by *R. solani*. It was suspected that certain antibiotic fungi or bacteria were at least partly responsible for such decrease. In fact, certain exploratory experiments indicated that the cornmeal amendment greatly increased the growth of *Trichoderma lignorum* (Tode) Harz, which Bisby, James, and Timonin (1), Weindling (11), and others, have found to be antibiotic to *R. solani*.

Three experimental soil series, each consisting of two 1-liter flasks of natural soil artificially infested with *R. solani*, were prepared. Unsterilized cornmeal was mixed with the soil of two flasks of these series at the generous rate of 30 cc. of cornmeal to 1000 gm. of soil. One heaping teaspoonful of spores of *T. lignorum* was added to each flask of the cornmeal series, and also one to the series having no cornmeal. Fourteen microscope slides were buried in the soil of each of two experimental flasks of soil in each series, and two of these slides were carefully withdrawn from each flask every five days during

35 days and examined under the microscope. At the end of 35 days, the soil in the flasks was thoroughly stirred and reset with microscope slides for two extra examinations. The data obtained from this study are summarized in Table I, where the figures express the average estimated abundance of *R. solani* to *T. lignorum*, with 10 as the maximum.

TABLE I

PERSISTENCE* OF *Rhizoctonia solani* IN A NATURAL SOIL AS AFFECTED BY *Trichoderma lignorum* AND UNSTERILIZED CORNMEAL

| 5-day sequence | Soil–cornmeal | | *Trichoderma lignorum* | | | | Remarks |
|---|---|---|---|---|---|---|---|
| | | | Soil–cornmeal | | Soil | | |
| | R.** | T.** | R.** | T.** | R.** | T.** | |
| 1 | 0 | 10 | 0 | 10 | 10 | 0 | Note heavy growth of *Trichoderma* favored by cornmeal in first sequence |
| 2 | 1 | 9 | 1 | 9 | 9 | 1 | |
| 3 | 1 | 9 | Tr. | 5(5) | 8 | 2 | |
| 4 | Tr. | 8(2) | 1 | 9 | 9 | 1 | Both fungi show definite signs of senility |
| 5 | Tr. | 7(3) | 1 | 9 | 9 | 1 | |
| 6 | 3 | 7 | 1 | 8(1) | 9 | 1 | Both fungi disintegrating |
| 7 | 2 | 8 | 1 | 9 | 8 | 1(1) | |
| (Soil stirred and reset with microscope slides) | | | | | | | |
| 8 | 1 | 8(1) | 1 | 8(1) | 6 | (4) | |
| 9 | 6 | 4 | 8 | 2 | 8 | 1(1) | *Rhizoctonia* tends to recover better than *Trichoderma* but growth of both fungi is slight |

* *Indicated by average growth ratio of Rhizoctonia solani to Trichoderma lignorum on clean microscope slides buried at the beginning of the experiment. Other fungi indicated in brackets.*

** *R. solani and T. lignorum respectively.*

Apparently *T. lignorum* grew almost to the exclusion of *R. solani* in both cornmeal series, whereas *R. solani* was very abundant in the series lacking cornmeal. This general relationship persisted until the eighth sequence, but it must be noted that the first growth on the slides could tend to persist. The growth of *R. solani* on the reset slides in the eighth and ninth sequence seemed to be better than that of *T. lignorum*. It is significant that after about 15 days the hyphae of both fungi on the slides showed unmistakable signs of senility. *T. lignorum* tended to disintegrate and to produce many spores, whereas many of the cells of *R. solani* changed from their natural hyaline appearance to a yellow color and seemed to be empty. Apparently the development of R. *solani* can be reduced severely at first by an abundant growth of *T. lignorum*

favored by cornmeal, but after a few days *R. solani* seemed to persist as well as, or better than, *T. lignorum*. Finally, these data help to explain why Daines (4) obtained no significant reduction of the *Rhizoctonia* disease of potato in field soil by infesting it with *T. lignorum*.

*Experiment 2*

*Persistence of R. solani as Affected by Roots of Wheat and Potato*

The purpose of this experiment was to determine whether either living or dead roots of wheat or potato plants affect the persistence of *R. solani* in the soil. There were seven series of infested virgin soil held in 1-gal. crocks. In four of these series, the roots of wheat or of potato plants grown there were left in the soil or screened out, as required. There were no plants in the remaining three series. At successive 21-day periods, fresh roots from potato or wheat plants reared in another uninfested soil were added to the soil of two of the series without plants. Two glass slides were buried in each crock of the seven series. These were examined after 21 days and new ones were buried again. The estimated abundance of *R. solani* on slides is recorded in Table II.

TABLE II

PERSISTENCE* OF *Rhizoctonia solani* IN ARTIFICIALLY INFESTED NATURAL VIRGIN SOIL AS AFFECTED BY ROOTS OF WHEAT OR POTATO PLANTS DURING 63 DAYS

| Plants | Infested soil | | | | | | | Noninfested soil | |
|---|---|---|---|---|---|---|---|---|---|
| | Wheat | | Potato | | Absent** | | | Wheat | Potato |
| Roots | Left in | Taken out | Left in | Taken out | Control | Wheat | Potato | Left in | Left in |
| First planting | 5(3) | 5(3) | 5(6) | 4(6) | 5(5) | 4(4) | 5(6) | 0(6) | 0(6) |
| Second planting | 2(6) | 1(5) | 6(3) | 5(5) | 2(3) | 2(5) | 2(5) | 0(6) | 0(6) |
| Third planting | 1(6) | 1(6) | 4(5) | 4(6) | 0(4) | 0(4) | 1(4) | 0(5) | 0(4) |

** Indicated by rated (1–10) growth on two microscope slides buried in each of three soil containers. Comparative ratings for other fungi in brackets.*

*** Roots added from wheat or potato plants grown in noninfested soil.*

In the first planting, the new growth of *R. solani* was fairly abundant in the soils of all seven artificially infested series, regardless of the crop or other treatment. However, in the second sequence, after a period of 42 days, a decided decrease of the pathogen occurred in all series, except in those having potato plants. The persistence of *R. solani* in the third sequence obviously reached a low point in all units, except those having potato plants. There the rating for persistence was still almost as high as it was at the beginning of the experiment, regardless of whether the roots were taken out or left in. Therefore, it seemed that the presence of living or dead roots of wheat or potato

plants plays a very minor role, if any, in the persistence of *R. solani*. The root remains appeared to favor the abundance of other saprophytic fungi much more than that of *R. solani*.

*Experiment 3*

*Effect of Sucrose, Calcium Hydroxide, or Cornmeal Amendments on Persistence of R. solani*

The preceding experiment indicated that the susceptible potato host was of far greater importance in aiding the persistence of *R. solani* than were the dead or living roots of a nonsusceptible host. The purpose of the present experiment was to determine whether the potato plant aided the persistence of the fungus more than did certain amendments, such as sucrose and calcium hydroxide, which in a previous study (10) seemed to favor it. Cornmeal was included here for comparison, because this amendment had appeared to depress *R. solani* more than did sucrose.

Each of the three amendments just mentioned were mixed in six 1-gal. crocks of artificially infested virgin soil. Three of these were planted with potatoes and the stems were rated for disease every 21 days; a corresponding number had no crop. At each successive replanting period, the soil in all crocks was kept separate and stirred. Three Petri plates of the soil were taken from each of the crocks and a microscope slide was buried in each plate. Six days later the relative abundance of *R. solani* on each slide was rated. This was repeated for 10 21-day periods. In the 11th replanting, potatoes were planted in all crocks of each of the three amendments used, and also in the control, and rated for disease as usual.

It is obvious from the data in Table III that the disease rating was a much more reliable measure of the persistence of the pathogen than that which was obtained from buried glass slides. In the absence of the potato host, however, *R. solani* practically disappeared from the slides after about 63 days. At the end of the first 21 days, its presence on the slides was too erratic to warrant any conclusion as to the differential effect of the various amendments used. The better persistence of the pathogen on the slides buried in the soil producing living potato plants was probably a direct result of the presence of disease on the stems of the host. Apparently the pathogen persisted equally well in the sucrose, calcium hydroxide, cornmeal treatments, and control series. The variations recorded for the different soil treatments throughout the different tests might be expected. The disease ratings in the 11th replanting indicate that the pathogen was no longer active, and possibly nonexistent in the fallowed series and that it had very noticeably decreased in all the series having potato plants. Apparently the rate of its decline in the presence of the host may vary considerably in different experiments of this kind (10). Garrett (6) concluded that an adequate supply of nitrogen to stimulate fresh hyphae of *Ophiobolus graminis* Sacc. seemed necessary for the survival of this pathogen in infected wheat straw buried in the soil. He also recognized the suppressive

TABLE III

PERSISTENCE* OF *Rhizoctonia solani* IN A NATURAL VIRGIN SOIL AS AFFECTED BY CERTAIN AMENDMENTS** AND BY THE PRESENCE OR ABSENCE OF LIVING POTATO PLANTS

| 21-day sequence | Sucrose | | | Calcium hydroxide | | | Cornmeal | | | Control | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Plants | Fallow | Disease rating, % | Plants | Fallow | Disease rating, % | Plants | Fallow | Disease rating, % | Plants | Fallow | Disease rating, % |
| 1 | S | S | 28 | T | T | 37 | L | L | 69 | S | S | 39 |
| 2 | L | S | 64 | L | S | 44 | L | L | 71 | M | O | 81 |
| 3 | H | T | 46 | L | O | 61 | T | T | 67 | L | O | 71 |
| 4 | O | O | 46 | S | O | 80 | T | O | 71 | L | O | 80 |
| 5 | L | O | 57 | S | T | 44 | S | T | 75 | S | T | 70 |
| 6 | T | O | 59 | T | O | 40 | S | O | 37 | M | O | 37 |
| 7 | L | T | 57 | L | O | 59 | T | O | 31 | S | O | 60 |
| 8 | S | O | 60 | T | O | 56 | T | O | 14 | T | O | 46 |
| 9 | L | O | 36 | T | O | 6 | T | O | 11 | T | O | 9 |
| 10 | L | O | 17 | T | O | 7 | O | O | 7 | S | O | 21 |
| 11 | | (2)*** | 10 | | (O)*** | 7 | | (O)*** | 6 | | (O)*** | 11 |

* *Estimated (none, trace, slight, light, medium, heavy) growth, during six days, of the pathogen on three microscope slides in each crock of soil and by disease rating after 21 days on the plants.*

** *Grams per 2000 gm. of soil: sucrose, 20; calcium hydroxide, 3; cornmeal (sterilized), 60.*

*** *Disease rating on plants in fallow series.*

effects of the associated microflora. Fellows (5) thought that the abundance of *O. graminis* in experimental soil might vary somewhat according to the abundance and kind of microflora present and to the direct effects of ordinary environmental factors.

*Experiment 4*

*Influence of Different Plants on Persistence of R. solani*

Exploratory studies suggested that living potato roots might serve to indicate the persistence of the pathogen better than buried microscope slides, inasmuch as the roots are not parasitized and they permit, and perhaps favor, the growth of *R. solani* on them. Therefore, the differential effects of the living roots of potato, pea, bean, wheat, corn, flax, and oat on persistence were compared. Two cultures, Nos. 76 and 106X, both pathogenic to potato, pea, bean, and flax, were used for the purpose mentioned.

Virgin soil was artificially infested as usual, planted at successive 21-day periods, and the soil in each container was kept from contaminating that of another during the entire experiment. A liberal representative sample of roots from the different kinds of plants was examined under the microscope every 21 days, and rated for relative abundance of the fungus in each instance. This rated abundance was recorded as none, trace, slight, light, medium, or heavy. Disease rating and degree of root colonizing were recorded for the eighth sequence on potato planted in all series.

As usual in the freshly infested soil, the roots of the various test plants in the first planting were heavily infested with the pathogen (Table IV). In the second sequence, the pathogen was relatively abundant on the roots of potato but noticeably less on the roots of the other hosts. The roots of wheat, oat, and corn were practically free of the pathogen in the third planting. In

TABLE IV

PERSISTENCE* OF *Rhizoctonia solani* IN A NATURAL, VIRGIN SOIL, AS AFFECTED BY DIFFERENT CROPS

| 21-day sequence | Potato | | Pea | | Bean | | Wheat | | Corn | | Flax | | Oats | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 106X | 76 | 106X | 76 | 106X | 76 | 106X | 76 | 106X | 76 | 106X | 76 | 106X | 76 |
| 1 | M | M | O | O | T | S | T | S | T | S | M | M | T | O |
| 2 | L | H | T | O | T | T | O | S | T | O | T | O | O | O |
| 3 | M | M | O | S | O | S | O | O | O | O | T | T | O | O |
| 4 | M | M | S | O | O | O | O | O | O | O | O | T | O | O |
| 5 | M | M | S | O | S | O | O | O | T | O | O | T | O | O |
| 6 | M | M | S | S | O | O | O | O | O | O | T | T | O | O |
| 7 | L | M | O | O | O | O | O | O | O | O | O | T | O | O |
| 8 | (40) | (43) | (7) | (14) | (15) | (23) | (0) | (0) | (0) | (0) | (8) | (9) | (0) | (0) |
| | L | M | T | O | O | T | O | O | O | O | O | O | O | O |

* *Indicated by (none, trace, slight, light, medium, or heavy) rated abundance on roots of test plants and by disease rating, in brackets, on potato stems, and by root colonization in eighth planting.*

contrast, the roots of flax, pea, and bean, the stems of which were slightly parasitized, were colonized irregularly in this and in the next four plantings. The stems of potato continued to be heavily parasitized throughout the eight sequences, and hyphae of the pathogen were relatively abundant on the roots. Potato planted in the pea, bean, and flax series in the eighth sequence had a light disease rating, but only a trace of, or no hyphae, on the roots. Potato in the corn, wheat, and oat series apparently was not parasitized, and there were no hyphae of the pathogen on the roots. Apparently the presence of hyphae of *R. solani* on the roots of the various hosts used in this experiment was largely incidental and not due to a nutritional relationship. In other words, it seems that the pathogen would not be on the roots, which it does not attack, unless it persisted by parasitizing the stems.

*Experiment 5*

*Persistence of R. solani as Affected by Different Host and Nonhost Plants*

The evidence from Experiment 4 indicated that the persistence of *R. solani* in the soil could be measured much more accurately by disease rating on the stems of the test plants than by degree of root colonization. No nutritional relationship between root and *R. solani* was apparent for wheat, corn, and oat, and, unfortunately, no evidence could be obtained on this point for potato, bean, pea, and flax, because the stems of those hosts were parasitized by both the pathogenic cultures used. The aim of this experiment was to compare again the effect on the persistence of the pathogen of various hosts used in Experiment 4, including barley. This time two cultures were used, namely, No. 76, which was virulent on potato as well as on bean, pea, and flax, and another culture, No. 32, which in many tests had not produced lesions on potato stems, although it had been consistently pathogenic to pea and bean.

Virgin soil was heavily and uniformly infested with one or the other of these cultures of *R. solani* as required, and put into 1-liter Erlenmeyer flasks and planted with potato, bean, pea, flax, wheat, barley, or oat, as necessary. There were three flasks for each test and the soil in each one was very carefully kept from contaminating that of the others throughout. Plants were taken up every 21 days, the stems rated for disease, a representative sample of roots was rated for hyphae of the pathogen, and then the flasks were replanted. Beginning at the second replanting, a freshly infested control series was planted with potato each time for comparison. The data obtained are presented in Table V.

On potato, culture No. 32 did not produce a lesion during the six replantings, although a mere trace of the fungus continued on the roots. In contrast, culture No. 76 produced severe disease in all plantings, and its hyphae were fairly abundant on the roots.

On bean, both cultures, despite fortuituous variations, were about equally virulent and also colonized the roots to the same extent. Although culture No. 32 seemed more virulent than culture No. 76 on pea, there was no reliable difference in degree of root colonization.

TABLE V

PERSISTENCE* OF *Rhizoctonia solani* IN A VIRGIN SOIL AS AFFECTED BY DIFFERENT HOST AND NONHOST PLANTS

| Sequence** | Culture number | Potato | | Bean | | Pea | | Flax | | Wheat | | Barley | | Oat | | Control (potato) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Root | DR, % | Root | DR, % | Root | DR, % | Root | DR, % | Root | DR, % | Root | DR, % | Root | DR, % | Root | DR, % |
| 1 | 76 | H | 86 | M | 21 | S | 0 | H | — | H | 0 | T | 13 | L | 0 | | |
| | 32 | T | 0 | T | 16 | T | 0 | M | — | O | 0 | O | 0 | O | 0 | | |
| 2 | 76 | M | 86 | L | 33 | O | 12 | H | — | L | 0 | O | 0 | T | 0 | H | 86 |
| | 32 | L | 0 | L | 86 | T | 16 | H | — | O | 0 | O | 0 | O | 0 | | |
| 3 | 76 | H | 71 | L | 43 | O | 4 | O | — | O | 0 | O | 0 | O | 0 | H | 86 |
| | 32 | O | 0 | S | 14 | S | 28 | O | — | O | 0 | O | 0 | O | 0 | | |
| 4 | 76 | H | 83 | L | 54 | S | 4 | T | — | O | 0 | T | 0 | S | 0 | H | 80 |
| | 32 | O | 0 | T | 28 | S | 86 | O | — | O | 0 | O | 0 | O | 0 | | |
| 5 | 76 | M | 86 | S | 0 | M | 0 | M | 0 | T | 0 | O | 0 | O | 0 | H | 86 |
| | 32 | T | 0 | O | 0 | O | 14 | T | 0 | O | 0 | O | 0 | O | 0 | | |
| Potato planted in all series | | | | | | | | | | | | | | | | | |
| 6 | 76 | L | 67 | L | 10 | O | 10 | T | 40 | O | 0 | O | 0 | O | 0 | H | 86 |

* *Indicated by rated (none, trace, slight, light, medium, or heavy) growth of pathogen on roots of different hosts, and also by disease-rating (DR) on their stems.*

** *Replanted every 21 days and soil of each container kept carefully isolated.*

Flax was severely parasitized by both cultures in the first four plantings, and hyphae of the pathogen were fairly abundant on the roots in all but one of the five tests.

Wheat, barley, and oat plants were not parasitized and their roots were not colonized, except slightly in the first two plantings.

In all the different plant series, the degree of infestation on the roots by *R. solani* was directly associated with the degree of parasitism as expressed on the stems. In the fresh control, culture No. 76 produced severe disease on potato and always colonized the roots heavily.

The data in Table V show that the heavy soil infestation disappeared rapidly in the presence of living nonhost plants, whereas living host plants, especially potato, extended its persistence. On the basis of the data from culture No. 32, which is nonpathogenic to potato, the persistence of the pathogen was prolonged by virtue of disease produced and not by nutrition provided by the living roots.

*Experiment 6*

*Persistence of R. solani in Soil in Field Through the Winter Period*

An experiment (10) in 1947 indicated that when there was severe disease on potato produced in September there was relatively slight disease in the following April and practically none where there had been little or no disease. The results of the present experiments Nos. 1, 2, 3, 4, and 5 indicate that, despite a heavy original infestation of the soil with *R. solani* it decreased rapidly in the absence of host plants. If the pathogen usually declines in the soil, despite the presence of a susceptible host like potato (Tables III and V), it may be that the main danger to soil infestation in the following year comes from the pathogen being carried to the soil on plant material, or from sclerotia produced in the soil late in the previous year. Apparently our knowledge on this aspect of the problem is very deficient.

The soil containers in the present experiment were 1-gal. crocks. These were filled during late September with natural field or virgin soil of optimum moisture content, as required, artificially infested with a virulent culture of *R. solani*, and left in a covered trench in the field until needed for testing.

On Sept. 28 three of these crocks of soil were taken to the laboratory, where 10 disinfected potato sets were planted in each of them. After an incubation period of 21 days at 61° F. the stems of these potato plants were examined and rated for disease, and also the surface of their roots for the presence of the pathogen. Similarly, plantings were made in another three crocks, brought from the field late in October, November, December, January, February, and March, and at the beginning of June. Each time the moisture content of the soil was brought to optimum.

The data obtained from the experiment just outlined are presented in Table VI. The disease was very severe on the stems and the pathogen was abundant on the surface of the roots in the initial planting. However the disease ratings and degree of root colonizing recorded for the late October,

TABLE VI

PERSISTENCE* OF A HEAVY ARTIFICIAL SOIL INFESTATION OF *Rhizoctonia solani* UNDER FIELD CONDITIONS THROUGH WINTER

| Date of test | Virgin soil | | Cultivated soil | | Control soil |
|---|---|---|---|---|---|
| | Root** infestation | Disease rating, % | Root** infestation | Disease rating, % | Disease rating, % |
| September (1949) | — | 87 | — | 71 | 0 |
| October | L | 25 | S | 16 | — |
| November | S | 23 | S | 17 | — |
| December | T | 38 | S | 40 | — |
| January (1950) | L | 20 | S | 20 | — |
| February | S | 20 | T | 6 | — |
| March | O | 6 | O | 3 | — |
| June | O | Trace | O | Trace | 0 |

** Determined by average disease rating at 61° F. and by root infestation on monthly plantings of potatoes.*

*** The ratings (none, trace, slight, light, medium, or heavy) were made on roots from each of three disease-free plants in each of three crocks.*

and subsequent plantings including those in February, were distinctly less than those of the initial test. The pathogen seemed to remain fairly constant in the cooling soil, which became frozen during mid-November and thawed in early March. The disease severity soon declined from a slight amount in the late March test to a trace in early June, at which time the pathogen was practically absent from the roots of the host. These data indicate that in the absence of a host an initially heavy infestation of the soil in late September can, under field conditions and favorable moisture, decline to a very low point or disappear by the following May or June.

## Discussion

The three different methods employed for measuring the persistence of *R. solani* in the soil were: plant disease rating on a susceptible host; root colonization of indicator plants in the soil; and observed growth of the fungus on clean microscope slides buried in the experimental soil. The two latter methods, although direct and relatively simple, were found to be too unreliable for detecting even large differences in soil infestation. The first method mentioned, although indirect and obviously much more elaborate than the others, proved to be reasonably reliable and sensitive (Tables III and IV).

The part played in the persistence of *R. solani* by associated antibiotic fungi and bacteria, by living and dead roots of plants, and by various organic and inorganic supplements in a natural soil medium, offers much scope for speculation and experiment. These aspects were studied in the first three experiments. The data from Experiment No. 1 seem to prove beyond reasonable doubt that, when *T. lignorum* is nourished by cornmeal added to the soil, it can practically prevent the growth of *R. solani* for a relatively short time.

However, the pathogen grew well when cornmeal was omitted. Apparently relatively few soil-inhabiting fungi or bacteria are strongly antagonistic to this pathogen in an untreated soil medium (9). Obviously the growth of the pathogen on the buried glass slides reflects mainly the antibiotic effect, or the lack of it, at the beginning of the experiment, but it does not reveal its duration. The fresh growth on the glass slide, of hyphae of both *R. solani* and *T. lignorum*, which soon became senile and progressively deteriorated, seems to emphasize a very important aspect in the biology of the persistence of fungi and bacteria in a natural soil. Broadfoot (3) found that, in both sterilized and natural soil, the disease produced by *O. graminis* declined very rapidly and he assumed that this was caused entirely by the antibiotic microflora. Although the new growth of *R. solani* and *T. lignorum* on the slides in the freshly stirred soil of the eighth and ninth sequences was very light in comparison with that in the first one, the indications were that *R. solani* persisted in this soil as well as, or better than, *T. lignorum* did, despite the addition of cornmeal and the observed transitory antibiotic consequences. Apparently (Table II) neither living nor dead roots of wheat or potato affected the persistence of *R. solani* beyond its normal behavior in untreated, natural soil. Although living roots are not attacked, possibly these and the decaying ones might nourish *R. solani* as well as other microorganisms, including the antibiotic ones, without perceptibly affecting its normal persistence. The data from Table III, and from an earlier study (10), show that *R. solani* persisted as well in natural black soil producing potato plants as it did when sucrose, calcium hydroxide, or cornmeal were added. Blair (2), as a result of his experiments, suggested the necessity of a food-potential for the saprophytic growth of *R. solani* through the soil. He found that on buried glass slides, owing chiefly to the competitive effect of associated microorganisms, the growth was depressed by the addition to the soil of ground wheat straw or dried grass.

That parasitic nutrition is consistently of great importance in the persistence of *R. solani* in the soil is revealed by the data in Tables IV and V. In the presence of wheat, oat, barley, and corn plants the isolates used soon disappeared despite the heavy original soil infestation, but when susceptible plants, such as potato, bean, pea, or flax, were present, *R. solani* persisted for about 170 days or longer, and finally seemed to disappear (Tables IV and V). Further evidence of the importance of the parasitic nutrition to the survival of *R. solani* in soil is the fact that pathogenic strains of this fungus have been consistently absent from the virgin black prairie soil used in these and related studies. Padwick (8) found in casein and a bacterial culture accessory growth substances for *O. graminis* which he thought might be important in maintaining the balance of microorganisms in the soil. The behavior of *R. solani* just indicated need not be taken to minimize the importance of growth factors in the sense suggested by Padwick. The importance of growth factors to various soil-inhabiting bacteria has been shown by Lochhead and Chase (7) and

by others. Finally, if the pathogen soon disappears from a soil lacking suitable hosts (9 and 10), then the resting sclerotia or mycelia of virulent races formed in the soil or on plant parts late in the preceding season become very important in any disease control measure.

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